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CORONARY DISEASE ASSOCIATED WITH A SHORT PR INTERVAL AND A PROLONGED QRS

A CASE REPORT

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THE occurrence of a short PR interval with a prolonged QRS has been described many times since it was first reported over twenty-five years ago. Much controversy has arisen as to the mechanism of its production, and there is still considerable dispute. For example, in 1930 Paul White, with Wolff and Parkinson, made a thorough study of a series of cases and ascribed the condition to a functional bundle branch block, but emphasized its occurrence in healthy individuals with normal hearts. In 1938 White stated, ". . . the impulse in a given case may pass through a short circuit, perhaps a bundle of Kent (Kent, 1893) and sometimes through the usual circuit by way of the bundle of His (Holzman and Scherf, 1932)." In 1933 Wolferth and Wood put forth the explanation of an accessory conduction bundle, known as the bundle of Kent. In 1941 these last two authors presented additional cases in support of their original hypothesis. However, in 1938, Katz and Kaplan reported a case in a patient with severe cardiac damage caused by coronary sclerosis, which they contend renders quite unnecessary the theory of conduction over the bundle of Kent. They maintain that the mechanism consists of an upper nodal rhythm, with a spreading of the impulse through a preferential path in the junctional conduction tissue. Tung, in a report in 1936, concludes that functional bundle block probably of vagal origin occurs and is not a sign of heart disease. Pardee, in his textbook published in 1941, states that the most acceptable explanation is that suggested by Cossio, who contends that these complexes are due to the

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excitation of an overirritable focus in the ventricle by the auricular systole. Levine and Beeson, in 1941, described three cases of this type, which manifested paroxysms of ventricular tachycardia, rather than the auricular tachycardia ordinarily found. In one case acute coronary thrombosis was so closely simulated that an erroneous diagnosis was made. Levine mentions another case in which the patient developed paroxysms of auricular fibrillation and went on into congestive failure, even though there was no organic heart disease. Finally in 1942 Butterworth and Poindexter, by electrical means, were able to produce experimentally in the cat and in the dog, complexes of the short PR, wide QRS type at will. In these experiments the current generated by the auricular muscle was picked up by small silver electrodes, placed directly on the surface of the auricle. This current was then amplified several thousand times and used to stimulate one ventricle by means of similar electrodes placed on the ventricular epicardium or in the ventricular muscle. They concluded that the most logical explanation of the syndrome is a ventricular asynchronism, with premature contraction of one ventricle, activated by an abnormal conducting pathway. (Their experiments essentially produced another pathway from the auricle to the ventricle.)

Finally, in 1943, Wood, Wolferth, and Geckler made serial sections of the heart from a patient whose electrocardiogram had shown a short PR and a prolonged QRS (there was an inverted T-1). This patient had died during an attack of paroxysmal tachycardia. Gross examination of the heart was negative but histological studies revealed three muscular connections at the right lateral border between the right ventricle and the right oracle. Two of these bridged a small part of the ventricular cavity during their course. The authors concluded that the demonstration of such structures which should be capable of conducting an impulse to the ventricle furnishes adequate support for the hypothesis of an accessary AV conducting pathway to explain this electrocardiographic anomaly.

It is not intended in this report to enter into the controversy as to the mechanism of this interesting manifestation, but merely to present a case in which coronary disease occurred, without modifying the dominant rhythm of the short PR and prolonged QRS, but at the same time leaving unmistakable evidence of coronary disease. It might be well, however, to enumerate briefly just what is definitely known about the condition, omitting all theories and conjectures. (1) It occurs in the absence of organic heart disease. (2) It occurs most often in young people who are subject to paroxysmal tachycardia (auricular). (3) Occasionally paroxysmal fibrillation occurs in place of the tachycardia and very rarely ventricular tachycardia instead of auricular tachycardia. (4) It has been produced experimentally in animals by setting up an abnormal conduction pathway between the auricles and ventricles. (5) It has been abolished by the use of atropine, quinidine and exercise. It may return to normal rhythm spontaneously. (6) It occurs much more often in males than in females.

CASE REPORT

P. K., white male, aged 43 years, was admitted on Jan. 5, 1936, because of severe burns of the right thigh and leg. He had been discharged from the Army for epilepsy and

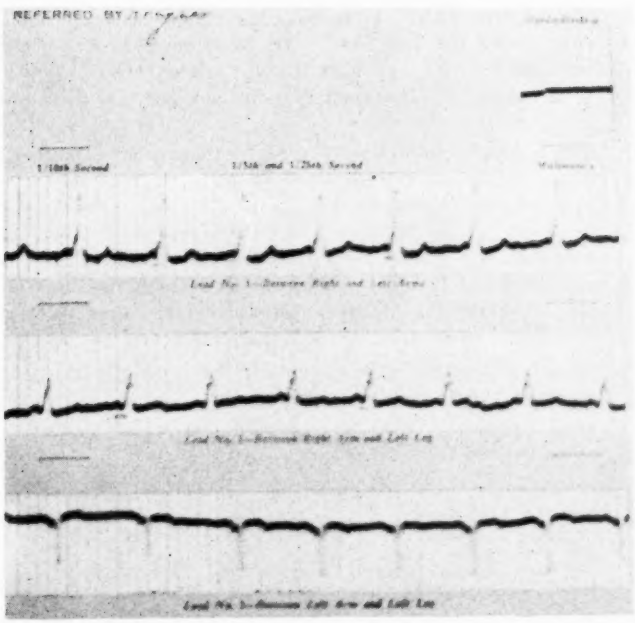

Fig. 1.—2/19/36. No cardiac complaints. Short PR and prolonged QRS.

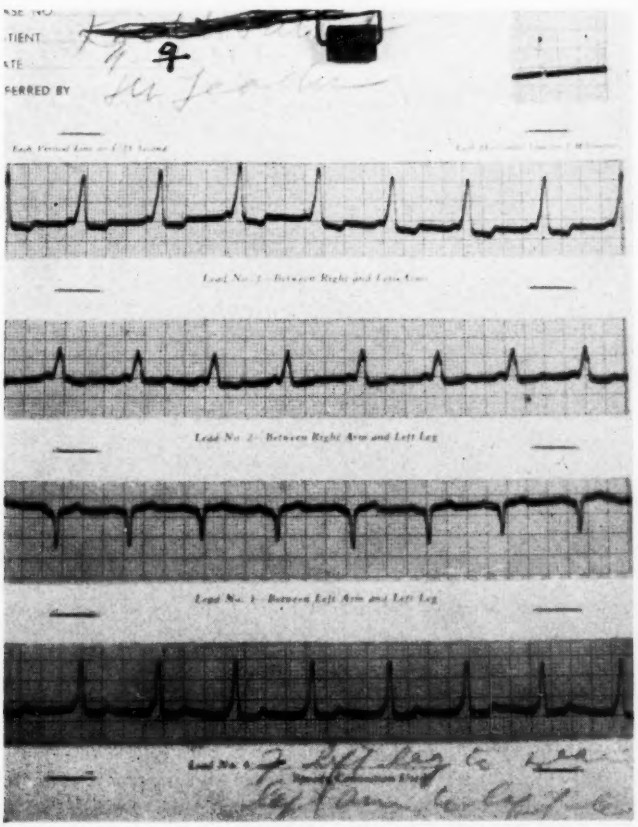

Fig. 2.—9/9/41. After first attack of severe precordial pain. T_1 inverted, ST_1 and ST_2 depressed.

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continued to have seizures ever since. Aside from his burns, physical examination revealed only hypertrophic arthritis of the left knee. An electrocardiogram revealed a short PR interval and prolonged QRS (see Fig. 1). The patient had no cardiac complaints, the blood pressure was 90/65, heart rate 70. The heart was not enlarged and there were no murmurs or thrills.

The patient made a satisfactory recovery from his burns, but continued to suffer from his arthritis and to have epileptic seizures. On Sept. 9, 1941, at 8:30 A.M., while stooping over, he was suddenly seized with severe precordial pain, which lasted for three or four minutes, and then subsided but soon became severe again. The severe pain came and went all that day and also the following day, when it was somewhat less severe. The pulse rose to 120 the first day, but soon decreased in rate. There was no temperature elevation. An electrocardiogram taken Sept. 9, 1941 (Fig. 2), revealed, besides the short PR interval and the wide QRS previously noted, a depression of ST-1 and ST-2 and an inversion of T-1. A cardiogram made Sept. 11, 1941, was essentially the same as the first, but a cardiogram taken Sept. 18, 1941 (Fig. 3), showed a diphasic T-2 and T-4.

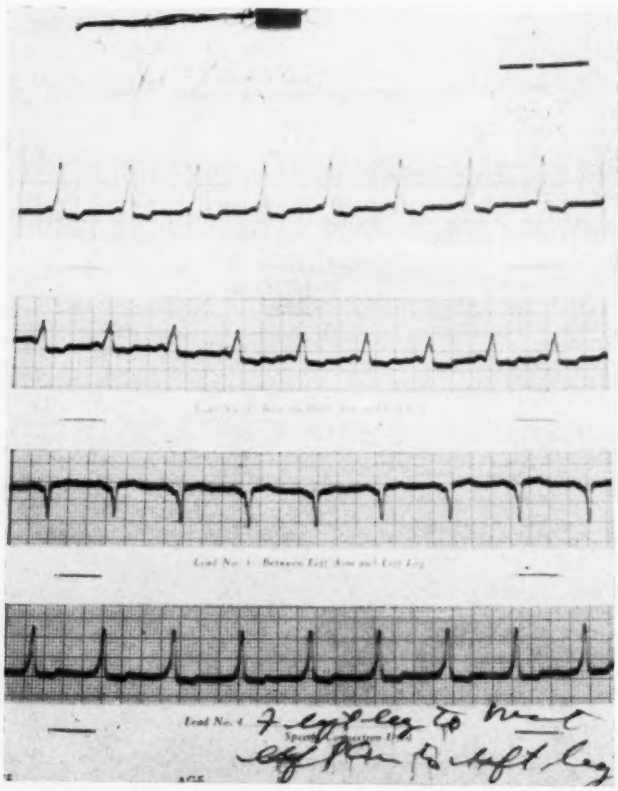

Fig. 3.—9/18/41. T_1 inverted, T_2 diphasic, and ST_1 and ST_2 depressed.

There was no pyrexia or leucocytosis and apparently the patient made an uneventful recovery. He gradually resumed his former activities; for a time he had no cardiac complaints aside from dyspnea on climbing stairs. On Dec. 1, 1941, however, while mopping the floor, he again experienced severe precordial pain, which lasted for one or two minutes, and then eased off. The severe pain was accompanied by a feeling of faintness so that he had to go to bed. Slight pain was still present the next day, but then disappeared. On December 25 he had another attack of severe precordial pain, lasting one or two minutes, but slight pain persisted all day. Following this he had an occasional "twinge" in the precordium, but continued to be up and around.

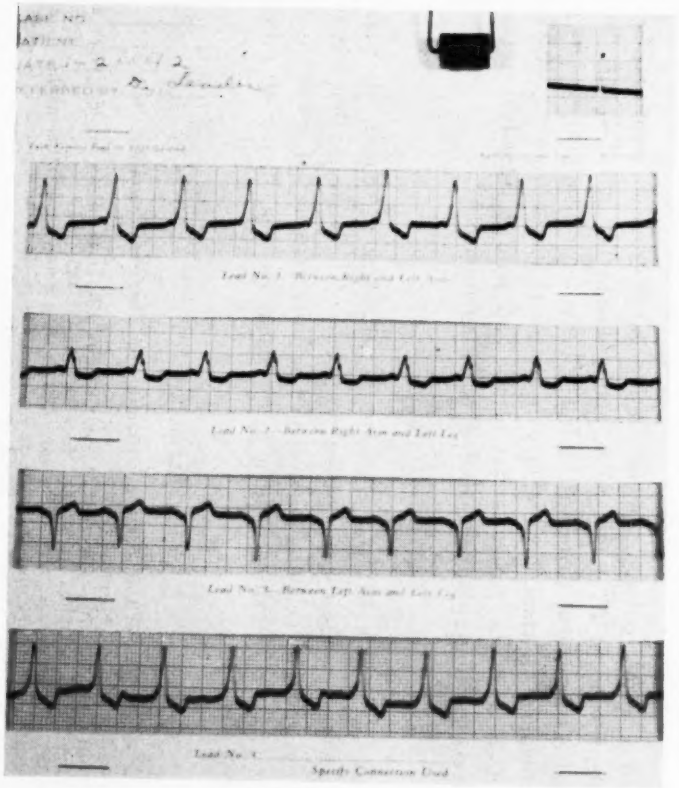

Fig. 4.

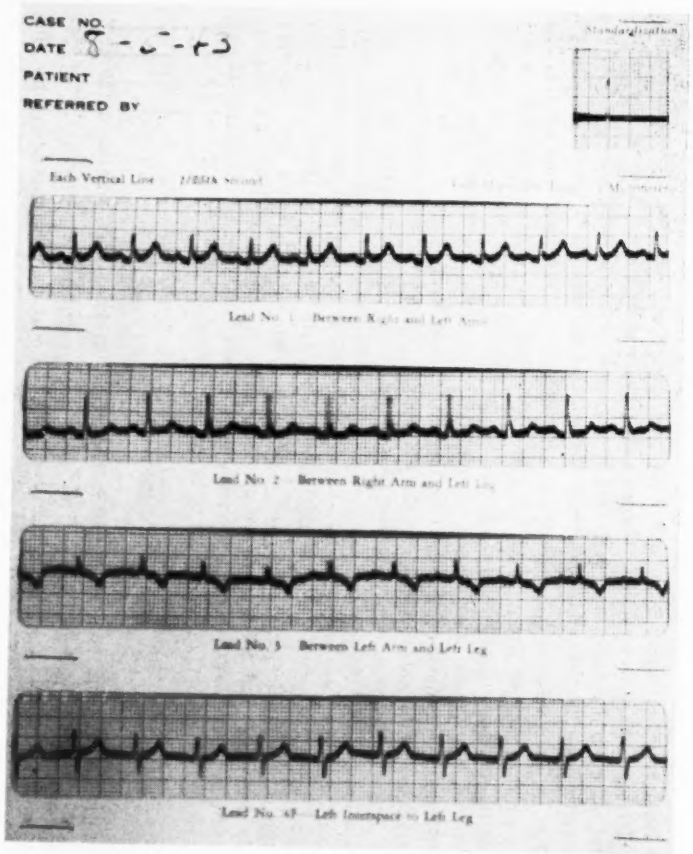

Fig. 5.

Fig. 4.—1/21/42. Four weeks after third attack of severe precordial pain. Deep inversion of T_1 and T_{4F}. Diphasic T_2 and depression of ST_1, ST_2, and ST_{4F}.

Fig. 5.—8/5/43. Normal PR and QRS, T-1 and T-2 upright, and ST-1 and ST-2 isoelectric.

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On Jan. 21, 1942, an electrocardiogram (Fig. 4) revealed a depression of the ST segments in Leads 1, 2, and 4F, deep inversion of the T-waves in Leads 1 and 4F and diphasic T-waves in Lead 2. The short PR and the wide QRS remained unchanged. Physical examination at this time revealed very little change. The blood pressure was 110/80, the heart rate 102, the white blood count was 11,350, with 70 per cent polymorphonuclears. An electrocardiogram taken Jan. 30, 1942, was essentially the same as the one taken on January 21.

On May 15, 1942, another cardiogram was taken which was similar to that of Jan. 21, 1942. His condition changed little during the next few months. During part of this period he was given theophylline. On March 11, 1943, he had another attack of sharp precordial pain but the temperature and pulse were normal, as were the white blood count and the sedimentation rate. No notable incident occurred during the next few months, but on Aug. 2, 1943, he developed epigastric pain which radiated to the precordium. This pain did not last long, but on Aug. 5, 1943, it recurred. He perspired freely, the sedimentation rate was 30 and the white blood count 16,300. The temperature rose to 102°, pulse to 118. An electrocardiogram, taken on Aug. 6, 1943 (Fig. 5), showed an entirely different pattern for him. Both the PR. and QRS were normal in duration and the QRS entirely normal in form. By Sept. 27, 1943, the cardiographic pattern had reverted to its usual form, but T-1 and T-2 were now upright and ST-1 and ST-2 isoelectric, whereas prior to August 6, 1943, T-1 was inverted, T-2 diphasic, and ST-1 and ST-2 depressed (Fig. 4). By Aug. 14, 1943, the patient was able to be up and around, and continues to be at this writing.

COMMENT

Coronary occlusion was strongly considered on the basis of the patient's first severe attack but could never be definitely established, although he developed definite evidence of chronic coronary disease as demonstrated by the tracing of Jan. 21, 1942 (Fig. 4). Again, on Aug. 6, 1943, when all the clinical manifestations pointed to coronary occlusion, the electrocardiogram suddenly reverted to a normal pattern. While the last cardiogram on record still shows a slight depression of ST-1 and ST-4, and diphasic T-1 and T-4, the changes in T-2 and ST-2 present prior to the last episode have disappeared. As mentioned previously, normal rhythm may be restored spontaneously. Just what connection, if any, the patient's last attack had with a transitory return to normal electrocardiographic pattern does not seem apparent.

SUMMARY

A case of a short PR and a prolonged QRS was presented. It was first recorded electrographically over seven years ago. The same rhythm was maintained, even though the patient developed definite evidence of severe coronary disease except for transitory return to a normal pattern during an attack of severe precordial pain.

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VERTIGO AND RELATED CONDITIONS*

A New Therapeutic Concept

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DIZZINESS, giddiness, lightheadedness, sensations of impending syncope, and actual syncope are well recognized as manifestations of cerebral anoxemia. It is also a fairly well accepted clinical fact that the most frequent cause of this anoxemia is cerebral ischemia, which is caused by cerebral arteriosclerosis with or without arterial hypertension. There has been some question as to whether true vertigo with its associated loss of equilibrium and sensation of rotation, with reference to external space, is ever caused by cerebral arteriosclerosis in the absence of thrombosis or rupture of a vessel, although it is admitted that dizziness and lightheadedness are common manifestations of this condition.[1] Actual vertigo, however, has been considered in some instances to be a manifestation of intrinsic vascular disease which may or may not affect the labyrinth.[2, 3] Lindsay,[4] in commenting on labyrinthine dropsy, has stated that vasomotor instability may be one of its more likely causes.

Also worthy of consideration is the likelihood that the various symptoms, as mentioned in the foregoing paragraph and which are manifest in the presence of cerebral arteriosclerosis, are caused by a decrease in volume of the blood flow with resultant tissue anoxia. This is analogous to the situation which occurs in angina pectoris and intermittent claudication when the vessels supplying the myocardium and lower extremities, respectively, are involved in occlusive arterial disease. Clinically, it would appear that one of the more important causes of vertigo, giddiness, and allied symptoms in the older age group manifesting arteriosclerosis is cerebral ischemia; this result is due to the sclerotic arterial vessels being insufficiently elastic to compensate for rapid changes of pressure. Especially is this true in regard to the intracranial arteries when the labyrinth is involved; the effects of gravity must be overcome. Also, it is known that only minute degrees of ischemia are necessary to produce symptoms.

The complaints of patients suffering from the syndromes under discussion vary from vertigo and syncope to ill-defined sensations of dizziness and lightheadedness. Characteristically, these symptoms are noted on arising from bed or when suddenly sitting up; they generally follow rapid changes of the position of the head, especially from dependency to the orthostatic position.

It is believed that because of the effects of gravity involved in the venous return from the brain, there may be a disparity between cerebral volume blood inflow and outflow in persons with impaired arterial circulation. This alteration of the normal balance of the circulation[5] cannot be appreciably improved in

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organic vascular disease by attempted improvement of the arterial circulation. However, the venous circulation of the intracranial contents is amenable to change inasmuch as the entire venous return is ultimately carried by the jugular veins. Application of certain physiologic principles involved in therapeutic venous occlusion for arterial disease of the extremities[6] and coronary arteries[7] to the cerebral circulation seems pertinent. By use of the plethysmograph it has been shown that following venous ligation in the normal, as well as the arterially deficient, extremity, there is produced an increased arterial pulsation which is caused by dilation of the vessels themselves.[8] It has been demonstrated experimentally that the effect of improved circulation to the part affected is not a humoral or nervous mechanism, but rather a mechanical one. Likewise, it has been shown that venous ligation, as well as the use of pneumatic tourniquet,[9] produces engorgement of the distal veins and venules which in turn is transmitted through the capillary bed to the arterial tree. This increase in diameter reduces the resistance, and the increased volume of blood flow results in the presence of fixed cardiac output.

For these reasons, it was believed that obstruction in the venous return of the intracranial contents might prove beneficial in the treatment of symptoms attendant upon inadequate arterial blood flow resulting from cerebral arteriosclerosis. With this object in mind, an elastic collar, ⅜ of an inch in width, was devised with a hook and eye attachment to permit ready removal; it is adjustable and may be tightened sufficiently to cause engorgement of the jugular veins without causing impairment of the arterial circulation. The collar was fitted into place at the base of the neck and well below the thyroid cartilage. The patients who were fitted with this device were advised to put it on fifteen minutes before arising from bed in the morning and to wear it continuously until nighttime when it was to be removed fifteen minutes after retiring.

Current conditions have made it impossible to obtain the clinical records of patients who were previously treated for a statistical tabulation; therefore, only one typical case report will be described.

F. F., male, aged 49 years, had complained of dizziness since October, 1942. The patient entered the hospital on Feb. 9, 1943, prior to which time he had been very active; he had practiced dentistry for twenty years prior to October, 1942. Previously, he had experienced no known arterial hypertension, renal disease, or other major illnesses, operations, or accidents. During the month of October, 1942, he noted the gradual onset of vertigo; this became quite severe within two weeks and necessitated his discontinuing his dental practice. The attacks consisted of recurrent sensations of loss of equilibrium, and subjective rotation, with reference to external space. These symptoms were noted most frequently upon arising from bed in the morning; they usually lasted four to five hours, then gradually subsided. Several of these episodes were induced by the rapid elevation of his head; other times they followed the tying of his shoelaces, or were incurred by leaning over a wash basin. Apart from the attacks of true vertigo, he experienced an almost continuous sensation of unsteadiness in his gait, insecurity while standing for prolonged periods of time, and, occasionally, fear of impending syncope as well as a slightly impaired memory for recent events. There was no associated tinnitus or hypacusis. The pressure was 168/108. The presence or absence of nystagmus at that time was unknown. The condition became progressively more severe, and the patient subsequently transferred to this hospital on Feb. 9, 1943.

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Upon physical examination, the patient was observed to be a well-developed, active, 49-year-old man, without edema, cyanosis, or abnormal increase of the venous pressure. Generalized peripheral arteriosclerosis was demonstrable in the temporal, radial, and pedal vessels. The blood pressure was 170/106. The carotid sinus pressure produced no change in the pulse rate, cardiac rhythm, or blood pressure, and was unassociated with any significant symptomatic response. The remainder of the physical examination was not remarkable.

The hemanalysis, urinalysis, and blood Kahn examinations were normal. The phenolsulfonphthalein excretion totaled 82 per cent in two hours by the intravenous method. Roentgen ray examination of the chest and intravenous pyelography were normal. The electrocardiogram was within normal limits. Following a three weeks' period of observation, during which time various medications were instituted and failed to alter the attacks of vertigo, the patient was fitted with the collar as previously described. Since this time he has been free of dizziness, his sense of equilibrium is normal, and he has returned to work; he now is able to stand for as long as eight hours without a recurrence of these symptoms. In addition to the definite improvement of his memory, he has noted a greatly increased sense of well-being for the first time since the onset of symptoms in October, 1942. The blood pressure has undergone no change since the employment of the collar.

Theoretically, the use of the collar would seem indicated in several conditions other than those under discussion; for example: cerebral arterial thrombosis during its early phase, postural hypotension,[10, 11] if unassociated with intrinsic arterial disease, and the amelioration of symptoms noted by pilots during rapid descent, the so-called "blackout" sensation.

The only untoward effects provoked by the wearing of this device have been occasional headaches. These apparently are induced by the increased intracranial pressure which results from having the collar too tight, and they are relieved by reducing the tension of the elastic. The use of the sliding adjuster on the collar, as is employed with garters, has proved to be a valuable contrivance for the maintenance of proper pressure. It has been observed that those patients with mild degrees of involvement are able to remove the collar in the afternoon without incurring a return of distressing symptoms; presumably, this is due to their having become adjusted to the orthostatic position by that time. No effects of carotid sinus stimulation have been induced by the wearing of the collar because of its position at the base of the neck.

As a general rule, the tension of the collar is adjusted so that a demonstrable venous pressure increase is obtained to the extent that venous engorgement is evident to the level of the mandibular angle. It has been found, however, that in fitting the collar to the individual person, the variations in the musculature of the neck, and other so far unknown factors, will require trials to be made with varying tensions before the optimum results are obtained in each instance.

It is realized that there is difficulty in evaluating any therapeutic procedure when the results are dependent upon purely subjective responses and are incapable of objective measurement. The possibility that examination of the retinal veins may be used as an index for establishing the degree of increased venous pressure is to be investigated. However, the accessibility of this device, its inexpensiveness, its lack of production of untoward effects, and its proved value, albeit of a purely subjective nature, in a relatively small group of persons, warrants its employment when other accepted methods of treatment for vertigo and related conditions have failed.

SUMMARY

1. The occurrence of vertigo, giddiness, lightheadedness, and syncope as manifestations of cerebral anoxemia resulting from cerebral arteriosclerosis is described.

2. The rationale of the application of certain principles of therapeutic venous occlusion for these symptoms is explained.

3. The clinical history of a patient who manifested the afore-mentioned syndrome and the results of therapy with partial jugular venous occlusion is reported.

4. Application of the principles involved in other conditions resulting from cerebral anoxemia is intimated.

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REVERSIBILITY OF SENSITIZATION OF ERYTHROCYTES

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IN PREVIOUS studies[1] we have sought to ascertain whether biologic agents which have been neutralized by antibody could be recovered in the active state. In these studies we took advantage of the fact that the proteolytic enzyme papain can apparently completely destroy the antibody function of the antibody molecule.[2] Thus, when the neutralized agents were submitted to digestion with this enzyme, it was found to be possible to recover them in the active form. By this method we have succeeded in quantitatively "reactivating" neutralized bacteriophage and botulinus toxin. Analogous experiments with intact, fully virulent pneumococci indicated that these organisms could be recovered in the virulent state even after having been completely sensitized, with all the antibody they could adsorb, to the extent of complete loss of infectivity for mice. We have also ascertained that the floccules of precipitate formed by the interaction of type specific polysaccharide of the pneumococcus with its homologous antibody could be dissolved by the action of the enzyme, with the liberation of the specific soluble substance into the medium. Finally, we have found that the pneumococcus "Quellung" reaction could be completely reversed by papain digestion, and the pneumococci thus denuded of their serum coating would again show "Quellung" reaction upon subsequent addition of fresh antibody.[3]

These studies bring direct supporting evidence to the concept that when an active biologic agent is "neutralized," the agent itself undergoes no essential change, except that it has become coated by antibody and thus cannot exert its characteristic action. When the antibody is removed, the original state of activity is restored.

We were interested in studying by this technique the effect of sensitization of erythrocytes by hemolytic antibody ("amboceptor"). Specifically, we wished to ascertain whether, by analogy with our previous findings, the susceptibility of sensitized erythrocytes to lytic action of complement could be abolished by the digestion with papain.

Technique.—The stock papain solution used in these experiments was prepared as follows: 4 Gm. of papain powder (Sargent) was shaken with 100 ml. of distilled water for one and one-half hours. It was then filtered through paper and diluted to 200 ml. This solution was kept at +7° C. under toluene and, when needed, an aliquot portion was activated by adding 0.1 ml. of 16 per cent solution of cystein hydrochloride for each ml. of enzyme solution, the hydrogen ion concentration adjusted to pH = 7.0-7.2, and the mixture incubated at least thirty minutes at 37° C. before use.

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The digestion of antibody was carried out by adding 0.1 ml. of the activated papain solution to 0.4 ml. of 4 per cent suspension of sensitized red cells and incubating for one hour at 37° C. The free enzyme was then removed by several cycles of washing with physiological salt solution in the angle centrifuge, and finally complement was added in the usual way to determine the presence or absence of sensitization.

Rabbit antisheep amboceptor and guinea pig complement were used throughout these experiments.

Results. In the preliminary experiments it was found that if 0.2 ml. of 4 per cent suspension of red cells, sensitized with from two to ten units of amboceptor, is subjected to digestion with papain, the cells lose their susceptibility to lysis by complement. To control this experiment, the sensitized cells were mixed with comparable amounts of papain which had not been activated, or with cysteine alone, and in either case the cells remained sensitive to complement.

It was noted in the above experiment that when the 0.2 ml. of 4 per cent red cells suspension had been sensitized with 10 units of amboceptor or less, the superambiant fluid contained no free amboceptor, indicating that it had all been absorbed by the erythrocytes. We were interested in repeating these experiments under conditions where the red cells were completely saturated with amboceptor, that is, when they had been sensitized by large excess of amboceptor. In order to accomplish this, it was first necessary to ascertain how much amboceptor a standard dose of red cells (0.2 ml. of 4 per cent suspension) could absorb. Thus 0.2 ml. of the red cells was mixed with 10, 20, 40, 80, and 160 units of amboceptor, respectively. The mixtures were then incubated at 37° C. for 30 minutes, centrifuged, and the supernates tested for residual amboceptor by serial dilution method. It was found that the cells present in 0.2 ml. of 4 per cent suspension took up approximately a maximum of 40 units of amboceptor.

The final experiment was then conducted as follows: To each of eleven tubes containing 0.2 ml. of 4 per cent suspension of red cells was added 80 units (large excess) of amboceptor (0.2 ml. of a 1:12.5 dilution). These mixtures were then centrifuged for 15 minutes in the angle centrifuge, and resuspended in 0.4 ml. of physiologic saline. To 8 of the tubes was added 0.1 ml. of activated papain, and the remaining tubes received, respectively, 0.1 ml. of saline, of papain solution which had not been activated, or of cysteine. The mixtures were reincubated for an hour at 37° C. and then washed with saline 5 times (to remove the added enzyme and other substances), and finally the sediments were each resuspended in 0.4 ml. of saline. Part of the tubes (1-4) in which the sensitized cells were digested with activated papain were tested for susceptibility to lysis by various amounts of complement, and part (508) were resensitized with various amounts of amboceptor and then tested with one unit of complement. As controls, the sensitized cells not subjected to papain digestion (9-11) were tested with one unit of complement. The results of the entire experiment are given in Table I.

This experiment indicates that when the red cells are sensitized to saturation with an excess of amboceptor, they become resistant to the action of comple-

TABLE I

DESENSITIZATION OF ERYTHROCYTES BY DIGESTION OF THE ANTIBODY WITH PAPAIN AND RESENSITIZATION BY FURTHER ADDITION OF AMBOCEPTOR

TUBE NO.	SENSITIZED CELLS	ENZYMES	TREATMENT	AMOUNT OF COMPLEMENT ADDED	RESULT	AMOUNT OF AMBOCEPTOR ADDED FOR RESENSITIZATION	AMOUNT OF COMPLEMENT ADDED	RESULT
	ml.	*0.1 ml.*	Incubated one hour at 37° C., then washed 5 times with saline and resuspended in 0.4 c.c. saline	*Units*		*Units*	*Units*	
1	0.4	Active Papain		1	No lysis	–	–	–
2	0.4	Active Papain		5	No lysis	–	–	–
3	0.4	Active Papain		10	No lysis	–	–	–
4	0.4	Active Papain		20	No lysis	–	–	–
5	0.4	Active Papain		–	–	1	1	Lysis
6	0.4	Active Papain		–	–	5	1	Lysis
7	0.4	Active Papain		–	–	10	1	Lysis
8	0.4	Active Papain		–	–	20	1	Lysis
9	0.4	Saline		1	Lysis	–	–	–
10	0.4	Papain not active		1	Lysis	–	–	–
11	0.4	Cysteine		1	Lysis	–	–	–

ment upon digestion of the antibody. This is true even when as much as 20 units of complement are used to induce hemolysis, indicating the completeness of the return to the unsensitized state. These cells can now be resensitized with even a minimum amount of amboceptor (1 unit) so that they become susceptible to hemolysis by but one unit of complement.

These results shed no direct light on the mechanism of the action of complement on the sensitized red cell. However, they do indicate that there has been apparently no permanent change in the cells as a result of the combination with hemolytic antibody as the cells revert to their original state of nonsusceptibility to complement upon the removal of the antibody from their surface.

SUMMARY

Red cells sensitized with an excess of amboceptor may be rendered completely resistant to complement, if the antibody is digested away by means of papain. These previously sensitized cells, after digestion by papain, can now be resensitized with a minimum of amboceptor.

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NOTE ON THE "DIGESTION" OF METAL IN THE STOMACH*

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THE "digestive" power of the stomach for metals has impressed us for a number of years during our experience with metal cannulae of all types used for the draining of gastrostomies or of pouches (Pavlov or Heidenhain) in dogs. The cannula consisted of a base plate and a tube. The base plate held the cannula in the stomach or pouch, and the tube was protruding to the outside, thus draining the stomach or the pouch. The cannulae were made of brass, ordinary steel, and stainless steel. They were made either of one piece, or the base plate was soldered, screwed, or braised to the tube. Soft, hard, or silver solder was used. A number of cannulae had been plated on the inside and outside with nickel, gold, or chromium. During periods varying between months and years a number of these cannulae dropped out of the stomachs or of the pouches because the solder from the base plate had dissolved; hard solder or silver braising were affected slower than soft solder, but dissolved nevertheless. We, therefore, constructed cannulae from one piece of metal in order to be certain that the gastric juice could not act on the joint between the plate and the tube. These cannulae lasted somewhat longer but were "digested" away eventually. In another number of dogs the base plate had dissolved entirely, or a section of the metal tube above the base plate had been "digested." The metal which apparently was destroyed easiest was stainless steel. On inquiry, the manufacturers told us that stainless steel had little resistance to weak mineral acids and that we would have been better off if we had used ordinary steel. In a number of cannulae a hole was digested through the tube, so that gastric juices leaked out around the tube.

Plating of the cannulae with different metals was not of much value. Apparently, small cracks or small bubbles which had been formed during the plating and which were not visible to the eye led to a diffusion of acid under the plating and to the eventual destruction of the cannula. The most resistant cannulae were those made of brass, but even they eventually became "digested." Apparently there are individual differences between dogs, because some animals might retain their cannulae for many years and in others they might be destroyed within a few months. We cannot explain this difference. The acidity in the pouches of our dogs was high; in many of them near the limits of the physiologic capacity of the stomach to secrete acid. In the case of the gastrostomy dogs, the acidity was naturally lower, because food, saliva, regurgitation, etc., tended to diminish it considerably. However, in these dogs "digestion" of the cannulae occurred.

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From the standpoint of the experimentalist it is to be welcomed that Wangensteen and his associates have developed a new cannula from plastic material. We hope that this cannula will stand up better in prolonged chronic experiments than our metal cannulae. We want to bring our experience to the knowledge of other workers in this field as a warning, and to the members of the medical profession in order to demonstrate how powerful the gastric juice is, not only to our ordinary food, but even to strong metal.

THE ADJUVANT EFFECT OF AEROSOL UPON THE GERMICIDAL ACTION OF CADMIUM CHLORIDE

ARTHUR F. COCA, M.D., PEARL RIVER, N. Y.

THE limitation of the fungal causes of ringworm to the epidermis and its appendages has suggested local treatment of this infection with fungicidal agents, and such treatment has been so frequently successful that one could be encouraged in the thought that the complete success of the method could be accomplished through a study of the several factors that conceivably are concerned in it.

In the present study the following pertinent factors have been considered:

1. The selected fungicidal agent should be one possessing a relatively low toxic or irritative property with respect to the cutaneous tissues.

2. The fungicidal agent should possess a sufficient property of diffusion into the cutaneous tissues and into the infective fungi; or if this property of diffusion is not sufficient in the agent itself, nonirritative adjuvants of such property must be sought.

3. If such an agent or mixture has been found it must be applied in a manner that allows sufficient *time* for effective penetration of the infected tissues and the infective fungi.

In 1935 the fungicidal property of cadmium chloride was strikingly shown when a heavy growth of mold that had resisted an oily antiseptic having a phenol-coefficient of 11 was entirely destroyed by a single application of a 10 per cent aqueous solution of cadmium chloride.

A one per cent solution of cadmium chloride in 20 per cent ethyl alcohol was then used in the treatment of a scaly eczema of the scalp and auditory canals. There was less scaling when the solution was applied daily but the eczematous lesions of the scalp were hardly influenced. The solution was not irritating.

When the marked wetting property of sodium dioctyl sulphosuccinate, Aerosol OT, became known, it seemed likely that its addition to the alcoholic solution of cadmium chloride would favor diffusion through the tissues and possibly penetration of the infective microorganisms by the cadmium salt.

One per cent of aerosol was used and it was found necessary to increase the concentration of the alcohol to 30 per cent in order to keep the mixture in solution.

The application of this solution daily to the scalp and ears in the above-mentioned case soon caused disappearance of the dandruff and healing of the eczema. Thereafter, recurrence of the condition could be prevented by applications at about three-day intervals.

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The same person was affected with recurring ringworm of the forearms and feet (athlete's foot) and these infections were entirely cured and later recurrence could be prevented through appropriate use of the alcoholic solution of cadmium chloride and aerosol. On one occasion the weekly use of the solution on the feet was discontinued for three weeks, after which time the early lesions of athlete's foot suddenly appeared between the second and third toes of the right foot accompanied with an intolerable itching. The condition healed in about three days after local applications of the solution were begun.

On the basis of these encouraging preliminary experiences, a number of cases of ringworm, affecting chiefly the feet, were treated with almost invariable success. Exceptionally resistant were infections of the plantar and palmar skin and the fingernail. Such cases required longer treatment, contact with the solution being maintained for 15 minutes or longer with gauze or cotton wet with the fluid. Clinical details of these cases will be reported elsewhere.

The first experiments designed to demonstrate the enhancement of the fungicidal action of cadmium chloride through the admixture of aerosol were carried out in an aqueous medium. The aerosol was used in a 50 per cent solution in propylene glycol, which was itself fungicidal. The addition of aerosol distinctly, though not markedly, increased the fungicidal action of the cadmium salt. When, however, the tests were carried out in a 20 per cent ethyl alcoholic medium the adjuvant action of aerosol was found to be remarkably strong. This experiment is illustrated in the table.

TABLE I

Illustrating the experiments that show the adjuvant effect of aerosol upon the germicidal action of cadmium chloride. The solutions of cadmium chloride and those of aerosol were made in 20 per cent ethyl alcohol.

The percentile figures of the two substances in the mixtures represent the final percentages obtaining after the mixtures were made. A constant quantity of the respective emulsified* microorganisms was transferred with bacteriological loop to the several mixtures and control fluids and after one-half hour a loopful of each of these was spread over the surface of a suitable nutrient agar slant. The slants were incubated at 37° C. The readings for survival were made after 48 hours.

$CdCl_2$ (½%) in each tube	Growth after 48 hours	$CdCl_2$ (¼%) in each tube	Growth after 48 hours
aerosol	Monilia albicans	aerosol	Monilia albicans
%		%	
1/16	none	1/16	none
1/32	none	1/32	none
1/64	none	1/64	none
1/128	none	1/128	2
1/256	none	1/256	few
1/512	1	1/512	good
0	good	0	good
Aerosol alone in 20% ethyl alcohol	Monilia albicans		
%			
½			
¼	3		
⅛	good		
1/16	good		
	heavy		

*The well-shaken emulsion of Monilia albicans was filtered through a silk fabric.

It is seen that whereas cadmium chloride alone in concentrations of ½ and ¼ per cent in 20 per cent alcohol was incapable of sterilizing suspensions of the microorganisms used in the tests, and whereas aerosol alone in the same solvent failed to kill these microorganisms in concentrations of $\frac{1}{16}$ per cent, the same two concentrations of cadmium chloride did sterilize the suspension when mixed with aerosol in concentrations as weak as $\frac{1}{256}$ per cent and $\frac{1}{64}$ per cent respectively.

TREATMENT AND CONTROL OF EPIDERMOPHYTOSIS AND BROMIDROSIS IN A STATE SCHOOL WITH CADMIUM CHLORIDE-AEROSOL SOLUTION

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EPIDERMOPHYTOSIS and bromidrosis are two clinical conditions occurring in institutions for which many remedies have been prescribed but for reasons discussed below few have been found entirely satisfactory. Recently, however, Lederle Laboratories of Pearl River, N. Y., made available to Letchworth Village, for investigational use, a cadmium chloride-aerosol solution which they had shown by laboratory and clinical tests to be fungicidal and antiodorant. This solution was given a clinical trial on 100 institutional cases. The results here recorded have shown it to be effective in the treatment and control of both epidermophytosis and bromidrosis, and most practical for institutional use.

This clinical trial was conducted among the adult female group at Letchworth Village, a state school under the New York State Department of Mental Hygiene, situated at Thiells, N. Y. Epidermophytosis and, to a lesser extent, bromidrosis are an ever present medical problem in this institution. These women, for the most part young and able-bodied, work during the day in various parts of the institution such as the laundry, the kitchens, and the infirmaries. Because of the nature of their work and the shoes worn their feet sweat or may be wet a good part of the time. Since such conditions favor the growth of the infectious fungi, it has not been unusual to find 25 per cent or more of patients in a cottage or dormitory afflicted with epidermophytosis of varying degrees of severity.

Prior to the use of the cadmium chloride-aerosol solution, various approved methods of treatment for epidermophytosis had been tried without reducing materially the incidence of the condition. These included such measures as the daily use of foot baths of 1 per cent sodium hypochlorite; daily foot baths of 1:5000 potassium permanganate solution; daily application of Whitfield's ointment U.S.P. full or half strength and in the most severe cases application of 1 per cent aqueous solution of gentian violet.

While effective in individual cases, each of these methods has disadvantages when applied to an institutional group. Foot baths are too time-consuming, particularly when one attendant has to treat a great number of cases in a limited time. Whitfield's ointment, even when used half strength, may cause erythema, burning, and itching in a certain number of cases. Gentian violet has been reserved for severe cases, for, while it gives highly satisfactory results clinically, it is difficult to remove from the hands, from floor or bed clothing and, therefore, is unsatisfactory because of the extra work it causes.

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The cases of epidermophytosis selected for treatment with cadmium chloride-aerosol solution were of two general types which we here designate as intertriginous and hyperkeratotic. The first group (intertriginous type) comprised 70 recent cases of mild, moderate, or extreme severity. In the mild and moderate cases, the skin between the toes was white and macerated with deep fissures occurring frequently. In severe cases, skin surfaces were raw and painful.

In these cases the feet were bathed daily, after which the solution was applied freely with a cotton-tipped applicator. In severe cases, the patients were confined to bed and treated with wet dressings of boric acid solution until they were able to bear the cadmium chloride-aerosol solution. Treatment was continued daily for from two to four weeks, although the itching was controlled and the drying effect of the solution observed after a few days. All cases were cured under this regime.

In two instances daily use of the cadmium solution caused reddening and itching of the skin. In such cases treatment was discontinued and a mild iodine ointment was used for a few days. Thereafter, treatment with cadmium chloride-aerosol solution was given two or three times a week.

The likelihood of reinfection from patients' shoes or from bathroom floors used by infected individuals was recognized. Since the actual fumigation of shoes as advocated by Beckman[1] is impractical for us at present, the shoes were sponged with cadmium chloride-aerosol solution and exposed to the sun during the day. Since these measures are probably inadequate, chief reliance for prevention of serious recurrence is placed upon the systematic weekly inspection of the patient's feet with resumption of treatment where recurrences appear.

The second group (hyperkeratotic type) comprised fourteen cases. In addition to showing some or all of the lesions described in the former group, the epidermis on the soles and sides of the feet was thickened, leathery and, in some instances, covered with greyish-white scales. In most of these cases the toenails were infected (onychomycosis). Skin specimens taken from six of these cases showed the presence of the fungous infection.

The technique of treatment used for the intertriginous type was found useless here. Instead, these patients were required to soak their feet in an adequate amount of the cadmium chloride-aerosol solution ten minutes daily after which the feet were massaged vigorously, thereby removing much of the infected epidermis. To date none of these patients has been cured, but they show definite improvement. The solution has been found nonirritating.

Treatment was applied to two cases diagnosed as epidermophytosis of the hands, the patients having previously had a severe infection of the feet. The hand condition healed completely after three weeks. Treatment was applied to one case of tinea circinata, with successful results.

For epidermophytosis the cadmium-aerosol solution has been found not only effective therapeutically, but safe, easy to apply, convenient, and, therefore, particularly suitable for institutional use where many patients may have to be treated in a limited time. The solution, applied in these cases over a considerable period of time, has caused no apparent damage to the clothing.

[1]Beckman: Treatment in General Practice, ed 4, W. B. Saunders Co., p. 793.

BROMIDROSIS

To try the value of cadmium-aerosol solution as a deodorant, sixteen female patients who suffered severely from bromidrosis, manifested as odorous underarm perspiration were selected. In all of these cases, even though shaving was performed at monthly intervals, the underarm odor was so strong that it would pervade a moderately sized room in a few minutes.

Each patient treated herself daily. After bathing, the solution was applied under arm with the finger tips. In all cases the results have been highly satisfactory and, where the patient is not doing heavy work, the odor is controlled completely. No complications have resulted. The solution is nonirritating to the skin and does not affect clothing deleteriously.

Similarly six cases were selected who were afflicted with bromidrosis of the feet. Each was put under treatment. The feet were bathed daily after which the solution was applied with a cotton-tipped applicator. In all cases the objectionable odor was completely controlled.

TABLE I

SUMMARY OF CLINICAL RESULTS IN 103 CASES WITH CADMIUM CHLORIDE-AEROSOL SOLUTION

DIAGNOSIS	NO. OF CASES	CURED	IMPROVED
I. Epidermophytosis			
A. Hyperkeratotic	14		14
B. Intertriginous			
1. Slight	38	38	
2. Moderate	26	26	
3. Severe	6	6	
II. Bromidrosis			*Controlled*
A. Underarm	12		12
B. Feet	6		6
III. Tinea circinata	1	1	

The cadmium chloride-aerosol solution submitted to the institution for investigational study has been in use for the past seven months. The incidence of cases of epidermophytosis during this time has been reduced markedly. Present indications are that we have here a therapeutic agent by means of which we can effectively reduce the severity and control the spread of epidermophytosis among a group of susceptible patients. Likewise, it is an efficient and convenient antiodorant.

MANUAL AND MECHANICAL RESUSCITATION IN EXPERIMENTAL ASPHYXIA

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THE purpose of this work is to determine the relative efficiency of manual and mechanical resuscitation with the type of instrument employed in our experiments. Sufficient reduction of oxygen intake results in severe damage to tissues and eventually in death. Complete restriction is fatal within several minutes. Conditions in which the body oxygen content is reduced are referred to as anoxia. Occasionally, the term is used interchangeably with that of asphyxia. However, the latter designation is applicable to those states which are associated with circulatory failure and retention of carbon dioxide.[1]

METHODS AND RESULTS OF INVESTIGATION

Asphyxia was produced by placing a tightly fitting rubber mask over the face of a dog and closing the open end with a rubber stopper. The animals were under sodium pentobarbital anesthesia.

In order to study gross changes in lungs and heart, asphyxia was induced in some animals by excising the anterior part of the chest wall. This produced an equalization of pleural and atmospheric pressures. After variable periods of asphyxia, resuscitation was administered either manually or mechanically with the McKesson resuscitator.

During the experiments, blood pressure tracings were obtained by means of a mercury manometer connected to the femoral artery. Respiratory activity was recorded by placing an inflated rubber tube over the chest and abdomen and connecting it to a tambour. In many instances bronchial respirations were obtained by connecting the tambour to the mask through the medium of a metal tube. Blood was drawn for quantitative analysis of oxygen and carbon dioxide from the femoral or carotid artery. Blood was drawn under oil. The analyses were made by the method of Van Slyke and Neill.[2]

To study the effects of resuscitation in carbon monoxide anoxia, the gas was administered to animals by a spirometer (with soda lime) which was filled with a mixture of 500 c.c. of carbon monoxide and four liters of oxygen. Carbon monoxide was obtained by the interaction of sulfuric and formic acids. The animals were allowed to breathe the mixture. When maximum respiratory effects were obtained, treatment was begun. This study was done to determine the rapidity of displacement of carbon monoxide by oxygen under various methods of resuscitation. Carbon monoxide analyses were done by the modified method of Van Slyke and Neill[3] on blood drawn from the femoral artery.

Asphyxia was produced by closing the open end of the mask and after varying intervals of complete cessation of respiratory movements, manual artificial

From the Toledo Hospital Institute of Medical Research, Toledo, Ohio.
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respiration was applied on four dogs in seven instances (Table I). Quantitative analyses for oxygen and carbon dioxide in arterial blood were made at regular intervals during the asphyxial and recovery periods and correlated with blood pressure and respiratory movements.

TABLE I

ASPHYXIA FOLLOWED BY MANUAL ARTIFICIAL RESPIRATION

NO. OF EXPERIMENT	TOTAL EXCLUSION OF AIR FOR		CESSATION OF RESPIRATORY MOVEMENTS FOR		OUTCOME
	MINUTES	SECONDS	MINUTES	SECONDS	
1	3	31		58	Died
2	4	31		17	Survived
3	5	24		40	Died
4	6	16	1	16	Died
5	6	19	1	2	Survived
6	6	24		46	Survived
7	6	40		23	Survived

Oxygen was quickly depleted from the arterial blood. The gas reached the low content of 0.17 to 1.41 volume per cent in 2 to 7.3 minutes of asphyxia. The total oxygen loss varied from 83.5 to 98 per cent of the preasphyxial level. The carbon dioxide gain was from 3.7 to 10.1 volume per cent. The total increase of carbon dioxide from the preasphyxial level varied from 6.5 to 25 per cent. These variations were due probably to the degree of narcosis, hemoglobin content, acid-base balance, etc. In less than two minutes after manual resuscitation was started, the oxygen content of the arterial blood reached the preasphyxial level. The carbon dioxide decreased below the preasphyxial content.

In instances of recovery, the blood pressure rose fairly quickly, in 18 to 34 seconds after artificial respiration was begun. In some cases when manual

TABLE II

ASPHYXIA FOLLOWED BY ARTIFICIAL RESPIRATION WITH THE RESUSCITATOR

NO. OF EXPERIMENT	TOTAL EXCLUSION OF AIR FOR		CESSATION OF RESPIRATORY MOVEMENTS		RESUSCITATION BEFORE MAXIMUM BLOOD PRESSURE IS REACHED
	MINUTES	SECONDS	MINUTES	SECONDS	SECONDS
1	3	6	1	31	45
2	4	6	--	26	24
3	5	14	1	19	38
4	6	4	1	--	81
5	6	23	1	5	18
6	6	24	--	54	27
7	6	30	1	40	46
8	6	57	1	22	29
9	7	57	2	45	--*
10	8	18	1	20	81
11	8	50	1	12	--*
12	9	2	--	25	33
13	9	45	1	55	--*
14	10	57	1	22	--*
15	11	22	1	37	--*
16	11	50	1	9	34
17	13	28	1	3	125
18	15	30	--	40	105

*Animals died. All others survived.

resuscitation was too brief for the degree of asphyxia, the blood pressure, after an initial rise, fell again. Subsequent manual resuscitation resulted in smaller blood pressure elevations.

Asphyxia was produced eighteen times in eleven dogs in one series of experiments. After variable periods in which complete cessation of respiratory movements was obtained, artificial respiration with the mechanical resuscitator delivering oxygen was instituted (Table II). Quantitative analyses for oxygen and carbon dioxide of arterial blood and records of blood pressure and respiratory movements were obtained.

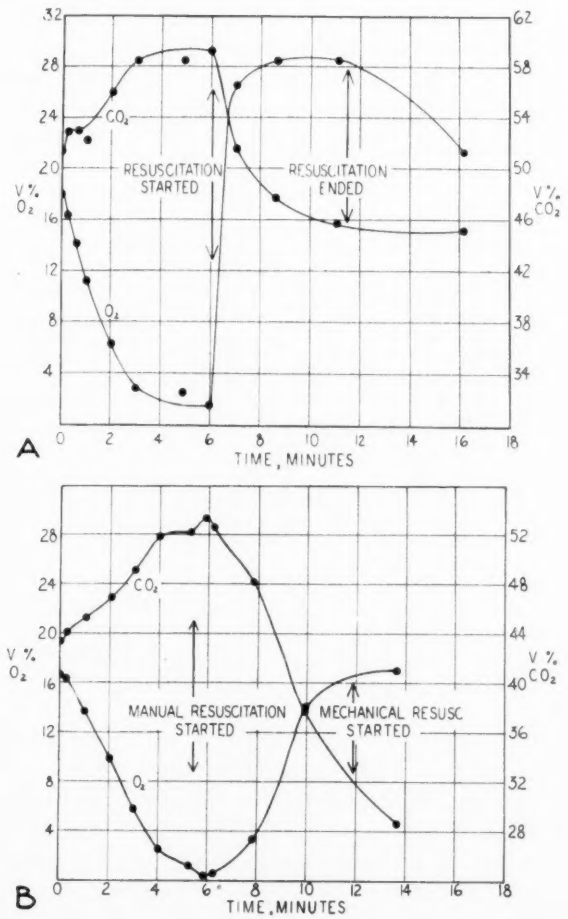

Fig.1.—*A*, Example of oxygen and carbon dioxide content in the arterial blood during asphyxia and after artificial respiration by a mechanical resuscitator. Note the rapid rise of oxygen to level greater than preasphyxial. Carbon dioxide dropped to a level below preasphyxial. The animal survived. *B*. Example of oxygen and carbon dioxide content in the arterial blood during asphyxia and after manual artificial respiration. The animal died. Note the considerable sluggishness in return to preasphyxial levels as compared with animal given mechanical resuscitation with oxygen in *A* in which the animal survived.

The loss of oxygen and gain in carbon dioxide during the asphyxial periods was essentially similar to that described in the experiments on manual resuscitation. In the stage of recovery, the return of oxygen was more rapid and it reached a higher concentration than in the preasphyxial period. The accumu-

lated carbon dioxide, after resuscitation had been begun, was lost rapidly. The restitution to preasphyxial levels was within the space of a minute. (Fig. 1.)

The maximum blood pressure was reached in surviving animals in from 18 to 105 seconds after resuscitation was begun. The pressure leveled off in the course of a few minutes. The respiratory movements returned to approximately preasphyxial type within the same period.

At no time during the life of any of the animals, did air or resuscitating gas fill the stomach. The organ became distended only when death occurred.

The next series of experiments was performed on seven dogs to obtain a comparison of three methods of removal of carbon monoxide: inhalation of air, inhalation of five per cent carbogen and resuscitation with oxygen. Carbon monoxide was removed most rapidly by inhalation of carbogen. Resuscitation with oxygen was almost as efficient. Inhalation of air lagged considerably behind the other methods. (Fig. 2.)

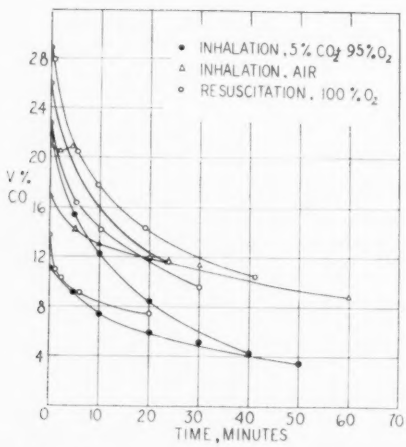

Fig. 2.—Determination of carbon monoxide in the arterial blood following treatment by inhalation of air, inhalation of carbogen, and resuscitation with oxygen. Note the greater efficiency of the latter two procedures.

The McKesson resuscitator and inhalator employed for artificial breathing in our experiments is of the following construction. The machine operates by virtue of compressed gas in the cylinder. The gas pressure in the cylinder in the case of oxygen is reduced to 50 pounds per square inch from a possible maximum of 2,000. Mechanical inhalation and expiration is accomplished by the action of gas under the reduced pressure on a piston which operates a bellows. The gas used to operate the piston is also delivered to the patient. The McKesson machine differs from other similar devices in the following: the volumes of gas delivered in inspiration and removed at expiration are fixed; a part of the expired air is reused; the balance of that air escapes through an escape valve which may be set at varying pressures; an additional fixed safety valve is provided for escape of the air. As a consequence of this mechanism, the lungs are never completely depleted of residual air. There are three controls. One valve controls the rate of resuscitation. Another regulates the volume of gas introduced and withdrawn from the lungs. The third valve controls the pres-

sure of the gas delivered to the patient (Fig. 3). There is also an inhalation device which allows the patient to inhale any volume of gas he desires up to 130 liters per minute.

In order to study the effects of the resuscitator, artificial respiration with the machine was administered to seven dogs under sodium pentobarbital anesthesia for periods varying from one to eight hours. Two of the animals were killed for the study of anatomic changes. One animal had been given artificial respiration for three hours and was killed immediately afterward. The other dog was given artificial respiration for six hours and killed twenty-four hours later. Gross examination of the organs revealed no abnormalities. Histologic study of the organs showed no abnormal changes outside of a mild hyperemia.

Analyses for oxygen and carbon dioxide of the arterial blood were done at regular intervals during the period of artificial respiration. The oxygen content showed an initial increase within the first five minutes, which varied from 3.2 to 6 volume per cent. The gas content in the arterial blood during the rest of the experiment continued more or less at this increased level.

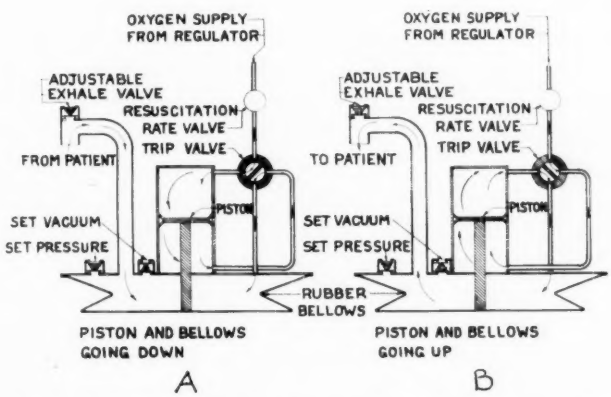

Fig. 3.—A schematic drawing of the McKesson resuscitator device. *A* indicates gas flow during patient's expiration. *B*, gas flow during patient's inspiration.

The carbon dioxide content of the arterial blood was decreased during the first 5 to 15 minutes of artificial respiration. The decrease varied from 1 to 5.5 volume per cent. The carbon dioxide content varied during the experiment from the preresuscitation level to that in the first several minutes. Since the resuscitator allows the rebreathing of part of the expired carbon dioxide, it would appear that the gas would be elevated in the blood. However, that was not true. It was apparent that the physiologic regulation limited the gas content. The rebreathing of part of the expired air takes the place of carbogen administration.

DISCUSSION AND SUMMARY

Acute asphyxia is a relatively rapid state which extends over a period which seldom exceeds fifteen minutes and which terminates in death.

Oxygen and carbon dioxide exchange constitutes the essential factor in these experiments. Within two to four minutes of acute asphyxia, the blood becomes almost completely devoid of oxygen. To insure recovery of the animal

in acute asphyxia, the oxygen must be restored with a rapidity comparable to its loss. Accumulation of carbon dioxide is simultaneous with loss of oxygen.

Methods in treatment of various degrees of asphyxia or anoxia may be classified into spontaneous and artificial. The former method presupposes the presence of adequate voluntary respiratory movements. The spontaneous method may be further subdivided into inhalation of air or oxygen or oxygen and carbon dioxide mixtures from an appropriate apparatus. The artificial method may be divided into manual resuscitation by various procedures such as Schaefer, Silvester, etc., and mechanical by several current devices. Since recovery of the animals was not attempted until all respiratory movements had ceased, the spontaneous method played no part in our asphyxial experiments.

Artificial manual resuscitation was performed on a number of animals. Recovery was obtained in a fairly large percentage. Recovery, however, was possible only when the duration of asphyxia was relatively short. The return of oxygen content of arterial blood was relatively slow. In some instances, death occurred after recovery from acute asphyxia with the oxygen content of the blood at normal preasphyxial level. It is believed that the cause of death was largely due to the sluggish return of oxygen and production of irreversible tissue damage. Artificial respiration with a mechanical device was responsible for a greater number of survivals. Survival was achieved after a relatively long period of asphyxia. Return of oxygen content to greater than preasphyxial levels was instantaneous.

In recovered animals, normal breathing and circulation was established after a relatively brief period of artificial respiration by either method. With the particular device employed in our experiments, there is a partial rebreathing of the expired carbon dioxide. This serves to add carbon dioxide to the resuscitating pure oxygen. There was no abnormal accumulation of carbon dioxide in the arterial blood nor were any disturbing physiologic effects noted. Mechanical devices have received condemnation from some investigators[4, 5] and warm support from others.[6-9] In our opinion, each method has its value depending upon conditions which call for their employment. When a mechanical device is not available, obviously the manual method must be utilized. On the other hand, availability of a tested mechanical device justifies its use. It is felt, however, that an intimate knowledge of the particular method and device is essential for its most effective use. In these experiments, no damage directly to the lung tissue or indirectly to other organs or tissues was observed even under exaggerated prolonged period of mechanical resuscitation.

CONCLUSIONS

1. Resuscitation with high percentage of oxygen administered in acute asphyxia by a mechanical device used in our experiments resulted in a more rapid return of oxygen content in the arterial blood than in manual resuscitation.

2. Prolonged resuscitation with the mechanical device did not produce abnormal functional or tissue changes in animals.

3. Inhalation of carbon dioxide and oxygen or resuscitation with oxygen and rebreathing of expired air with the mechanical device resulted in a rapid elimination of carbon monoxide.

4. Mechanical resuscitation in acute asphyxia with the device employed in our experiments is a more efficient procedure than manual resuscitation.

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BOTULISM FROM HOME-CANNED BEETS

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WITHIN the last three months, there have been brought to the attention of the Los Angeles City Health Department, two outbreaks of botulism caused by eating home-canned beets.

The case reported herein occurred around Jan. 6, 1944, and the investigation was made by Mr. R. S. Plunkett, R. S., of this department.

As the history of the cases, methods of canning of the beets, their subsequent handling, and the laboratory findings were in accord, we considered the case worthy of a detailed report.

On Jan. 6, 1944, Mrs. S., during the process of concocting some "borscht," removed seven whole beets and a pint of the red beet juice, from a jar of home-canned beets, and, after proper preparation and manipulation, this mixture was added to the other ingredients to complete the above "delicacy." The same day, about 6 P.M. Mrs. S., and her two grandchildren, aged 11 and 7 years, partook of this "borscht."

The following day, the 7-year-old child returned from school at noon complaining of terrific vomiting and retching and, as her grandmother described her, "with her eyes almost popped out." She was taken to the hospital and succumbed the following day with symptoms typical of botulism. The other child, aged 11, also returned from school the same day as the first, about 3 P.M., with similar symptoms; she was sent to the hospital and also died the following day. The grandmother was stricken about 6 P.M. with dizziness, violent vomiting, and blurred vision. She was sent to the hospital, given 15,000 units of botulinus antitoxin and eventually recovered.

Specimens of the beets and some of the borscht was submitted to the laboratory for examination. Animal inoculations, controlled with botulinus antitoxin A and B, showed that the beets and likewise the borscht, contained type A botulinus toxin. Anaerobic cultures were also made from the beets in order to isolate the causative organism. The beets showed a reaction of pH 6.9 which is practically neutral.

In describing the method used in canning the beets, Mrs. S. stated that she had first boiled the beets a few minutes until the skins would peel easily, then reboiled them and put them into the washed half gallon syrup container jars. From the fact that the label was still intact and in good condition on the jar, when received in the laboratory, it was assumed that very little washing and no sterilization was attempted on this container. The amount of cooking described for the beets was patently not sufficient to kill the botulinus spores that must have been present.

Too much stress cannot be laid on the necessity of thoroughly sterilizing all food and containers if attempting to do home canning. The above tragedy is just another example of the danger in the handling of home-canned products.

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THE WATERHOUSE-FRIDERICHSEN SYNDROME

Report of a Case Terminating in Recovery

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1st Lt. Lewis H. Bronstein, M.C.

THERE are numerous case reports of the Waterhouse-Friderichsen syndrome in the literature, particularly that dealing with pediatrics. Since the septicemia in the greatest majority of cases is due to the meningococcus, it is to be expected that, in the future, more cases will be reported in the adult population because of the increased incidence of this infection at present. Death has occurred in practically all the cases that have been reported to date. The picture is usually that of an individual who becomes acutely ill with a very high temperature and no physical findings. In a few hours, a marked purpuric eruption is noticed and within 24 to 48 hours, death occurs. Where cultures are taken, the meningococcus organism is the usual one that is recovered. Autopsy reveals hemorrhages into the adrenals as the outstanding finding. The fatal termination is blamed on the acute adrenal insufficiency that occurs with such hemorrhages because the terminal picture is that of circulatory collapse.

Before the days of the sulfonamides, there was little hope of saving such cases, even if the diagnosis were entertained because serum and antitoxin did not clear the body of the meningococci quickly enough to stop the overwhelming infection. The meningococci are so susceptible to the action of sulfonamides, however, that this possibility exists today. In addition, the availability of adrenal cortical extracts and plasma gives one the means of tiding a patient over the period of adrenal damage until sufficient sulfonamides are administered to clear the body of the organism.

In November, 1942, a soldier was admitted to the Station Hospital complaining of headache and fever of one day's duration. He had a sparse purpuric eruption on his body, a moderately rigid neck, and positive Kernig and Brudzinski signs. His temperature was 104°, pulse 120, respirations 30, and blood pressure 118/76. A spinal tap revealed a cloudy fluid which had 61 cells, 98 per cent of which were polymorphonuclear leucocytes. A few extracellular gram-negative diplococci were seen on smear. Meningococci were grown from this fluid and from the blood.

He was immediately given 6 Gm. of sulfadiazine by mouth followed by 1 Gm. every four hours. For the next twelve hours, until 10:00 P.M., he appeared to be doing well, with the temperature dropping. That evening, he seemed to be weak and it was noticed that although his temperature continued to drop his pulse and respirations were increasing. In addition, he was slightly cyanotic. The drug was continued and he seemed to hold his own during the night, though he did appear quite sick. About 6 A.M., he suddenly collapsed, with harsh

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breathing, shallow respirations, and a rapid and thready pulse. The Officer of the Day who had followed him throughout the night stated that the death was in the nature of a collapse rather than a toxic death.

An autopsy was performed several hours later. This showed the characteristic central nervous system changes associated with meningitis. There were petechial hemorrhages in the serous membranes and the skin. The lungs and the spleen were markedly congested. The kidneys showed changes of an acute glomerulonephritis and bilateral hemorrhagic necrosis. Both adrenal glands had large areas of hemorrhage in the cortices. Postmortem culture of the spinal fluid resulted in the growth of meningococci. No organisms were grown out of the heart blood.

Although many pathologic findings were seen on autopsy, it was felt that the marked adrenal destruction was responsible for his death. Since this was the first death we had had until then from meningococcic infection and, incidentally, the only one until now, though we have seen more than 80 cases of this disease, we decided to be ready for any subsequent similar case. In preparation for this, we kept adrenal cortex extract on hand and planned to use plasma and sulfonamides in conjunction with this in the next case that seemed to be a candidate for this complication. It was realized that all the medications would have to be administered very early in the course of the disease because the above autopsy showed the numerous pathologic changes that took place in a relatively short time.

The following case report is that of a soldier who was admitted to the hospital four months later with a meningococcic septicemia. He was treated with all the agents mentioned above and recovered after a short and stormy course.

CASE REPORT

This soldier was admitted to the Station Hospital on March 25, 1943, in a confused mental state so that adequate history could not be obtained at that time. He was quite drowsy but could be aroused to perform simple commands, to swallow tablets and fluids. He was evidently acutely ill with a temperature of 103°, pulse of 106, and respirations of 20.

Three days after admission, the following history was obtained from him. He went to bed on March 24, the evening before admission to the hospital, feeling perfectly well. About midnight he had a chill which lasted for several hours. His mouth felt very dry. He was able to fall asleep later and then awoke, feeling very dizzy and extremely weak. A severe headache had developed. This rapidly became worse and he reported for sick call. He had only the vaguest memory of events from that time until about 30 hours after admission.

Physical examination on admission revealed the following pertinent findings. The entire neck, upper chest, and all extremities, particularly the distal portions, were covered with purpuric lesions of all sizes. There were some lesions on the conjunctivae, which were injected. The throat was mildly hyperemic. There was no nuchal rigidity. The blood pressure was 60/40. The spleen was not felt. The Kernig sign was present to a slight degree.

It was felt that he had a fulminating meningococcus infection because sporadic cases had been occurring on the post during that time and because many cases were reported in Baltimore, which was his home. In addition, the rash was similar to that seen in previous cases of meningococcus infection. A blood culture was taken and a spinal tap was done. The fluid was clear and contained two cells, both of which were lymphocytes. The sugar content was 77 mg. per cent. No globulin was detected. Smear of the fluid did not reveal any organisms and none were grown out. The white blood cell count was 11,050 with 2 myelocytes, 22 juvenile granulocytes, 30 "stab" forms, and 39 segmented granulocytes. There were 5 lymphocytes and 2 mononuclear cells. The urine was positive for albumin and had an abundance of granular casts.

He was given 4 Gm. of sulfadiazine intravenously at 1:30 P.M. and 2 Gm. orally at 2:15, 6:00, and 10:00 P.M. Because he had an emesis of 500 c.c. of fluid shortly after the 10 P.M. dose, he was given 2 Gm. intravenously at 3:30 A.M. of March 26. Following this, he received 1 Gm. sulfadiazine orally every four hours. The blood sulfadiazine level at 7 A.M. of March 26, was 17.5 mg. per cent. The level on the morning of March 27, was 18.8 mg. per cent.

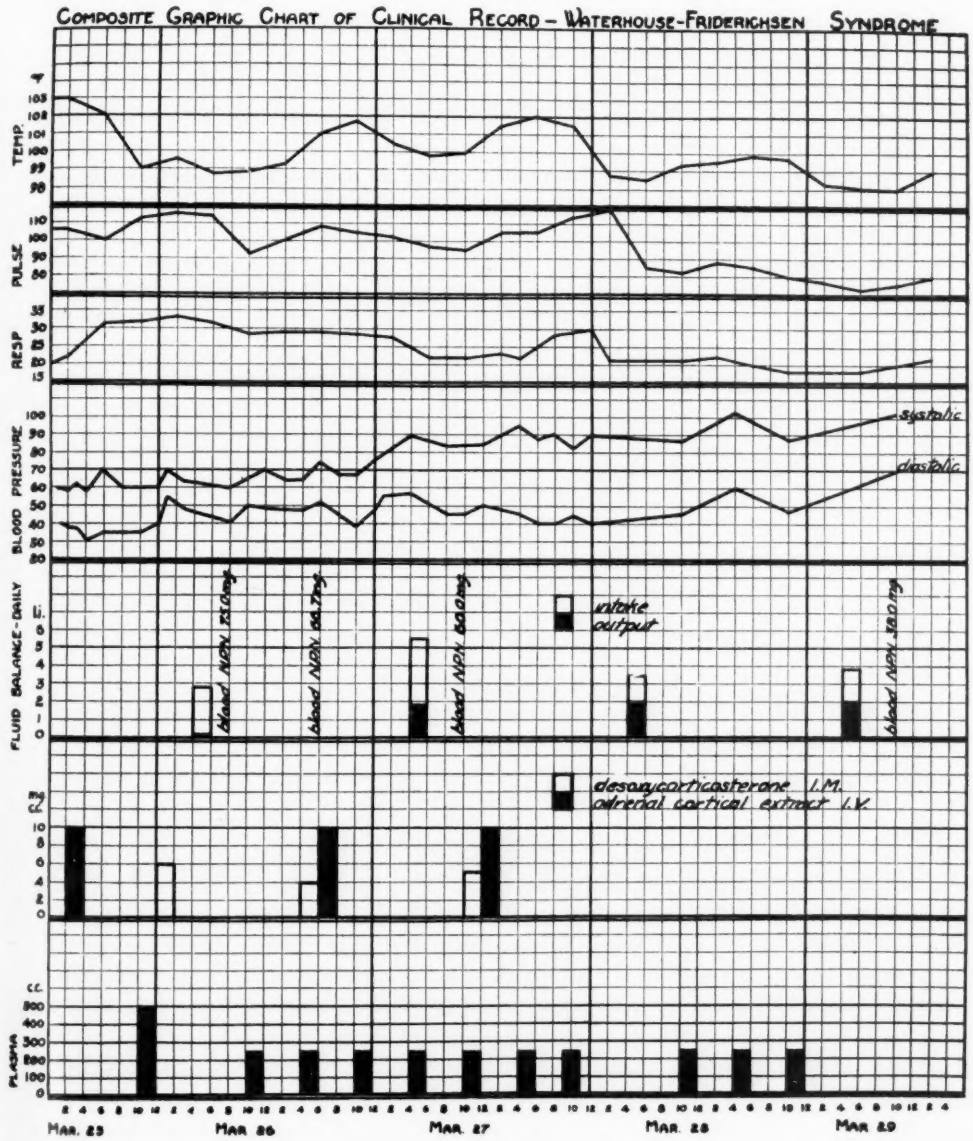

Fig. 1.

He took fluids well by mouth, except for the emesis mentioned above, so that the only fluids given intravenously were those contained in the plasma. The chart shows the amount of plasma which he received. This plasma is stored in 5 per cent glucose solution. The chart also shows the amount of adrenal cortex material given, as well as the time of administration, in relation to the other findings.

Between the time of admission and 1 A.M. of March 26, he had not voided, and only 5 c.c. of urine were removed from the bladder by catheterization. At 4 A.M., he was given 50 c.c. of 50 per cent glucose after hot loin packs had failed to cause further voiding. At 4:30 A.M., he passed 150 c.c. of urine. The chart records the intake and output for the preceding hours.

Chemical analysis of the blood was done at 8 A.M. on March 26 and showed nonprotein nitrogen 75, creatinine 2.6, sugar 93, and chlorides 388 mg. per cent. Examination at this time showed minimal nuchal rigidity, no change in the Kernig sign, and no new purpuric spots. The laboratory reported that a gram-negative diplococcus was growing in the blood culture. This was later shown to be a Group I meningococcus.

The blood chemistry determinations were repeated at 3 P.M. and showed nonprotein nitrogen 66.7, creatinine 2.4, sugar 137, and chlorides 370. He appeared mentally clear and looked to be in much better condition even though the blood pressure was low. In order to play safe, more plasma and adrenal cortical extract and synthetic desoxycorticosterone were given, as shown on the chart. In addition, he was given one salt tablet (2.25 gm.) every four hours as tolerated. He did not take many of these tablets because he became nauseated and they were discontinued after 24 hours. The blood chemistry performed on March 29 revealed the nonprotein nitrogen to be 38, the creatinine 1.5, the sugar 110, and the chlorides 396 mg. per cent.

The urinalysis showed normal findings on March 29 and remained normal throughout his stay in the hospital, although a trace of albumin was found on one occasion while he was still taking sulfadiazine. The white blood cell count returned to normal limits on March 31.

His condition gradually improved with a slow rise in blood pressure, which reached 118/80 on April 19, three weeks after the onset of his illness. This level was maintained throughout the convalescent period. The purpuric eruption had completely disappeared by March 30, but a bilateral conjunctivitis was noticed on that day. This cleared in 6 days with local therapy. It is to be noted that he was still receiving sulfadiazine during the development of the conjunctivitis. He received a total of 69 Gm. of sulfadiazine during his stay in the hospital.

On June 24, three months after the onset of his illness, the efficiency of his adrenal cortex was tested by means of the Cutler, Powers, Wilder test. Clinically there was no evidence of any insufficiency of this organ. The urine volume was 1295 c.c., the total chlorides was 121.4 mg. per cent. This is within the range of normal efficiency.

DISCUSSION

This case differs somewhat from the case which ended fatally. The soldier who died had a definite meningitis, with organisms recovered from the spinal fluid, as well as the blood. The sulfadiazine which he had received was apparently sufficient to sterilize the blood, but not the spinal fluid because postmortem cultures were negative for the heart blood but were positive for the fluid. His condition on admission, however, was apparently better than that of the second patient. This latter patient had much more purpura and a low blood pressure. In addition, he was more toxic as evidenced by the cerebral changes. The first man did not appear to be sicker than the average patient with meningitis that we have seen at this post. The second soldier had only a septicemia with the organism. His spinal fluid cytology and chemistry were normal and no organisms could be grown from it.

It is a matter of conjecture to discuss the amount of pathology in the adrenals that could have been present in the soldier who recovered. It must be assumed that he had more than mere edema of the gland on admission be-

cause of several factors. The blood nonprotein nitrogen was elevated. Despite the fact that he had received 50 Gm. of glucose intravenously during the preceding ten hours and had had practically no urinary output, the blood sugar level was only 93 mg. per cent. The blood pressure rose very slowly to his normal level. In some of the reports of meningococcus infection in the literature in which the patient is acutely ill with a low pressure, this value has returned to normal within one to two days. The slow rise in pressure cannot be attributed to his bed rest since he was in bed all the time that this rather steady rise was recorded. His pressure after he was up and about merely showed some fluctuations from his apparent normal of 118/80. The chloride determination does not help too much since the determination of the end point is not too definite and the change in the various determinations that were made did not correspond with the improvement noted in all the other findings. Unfortunately, we were not equipped to determine the sodium content of the blood.

For the first few weeks after he was allowed to be up and about, he complained of fatigability which could be attributed to his severe illness and his prolonged bed rest. His particular type of infection did not seem to be responsible for this. It was felt that it would be too risky to attempt to measure adrenal cortical function too early in his convalescence. This test was therefore done several days before he was discharged to duty. As is noted above, his response was normal and we must assume that, as far as could be determined, he had restitution of his cortical function.

We did not have too much adrenal cortex extract or desoxycorticosterone at hand to give the massive doses that are advocated for patients with acute cortical insufficiency. We tried, however, to use the material at hand as judiciously as possible. We always kept in mind the fact that he might not have too poor an adrenal function and that we would throw him into a water intoxication syndrome. Since plasma was readily available, we placed more reliance on that substance and gave him a fairly good amount of it. In addition, his fluid intake by mouth seemed to be quite adequate. It was our impression, during our vigil, that he obtained more benefit from the plasma than from the adrenal cortex material. We have no more evidence, however, than mere impression.

A study of the chart shows the very definite trend toward improvement in all his reactions. The temperature dropped gradually, as did also the pulse rate. The respirations became slower. The systolic and diastolic pressures rose with an increase in the pulse pressure. Although he had a very low urinary output during the first eighteen hours, this soon corrected itself. It is impossible to be definite about the role played by the intravenous glucose in starting his kidney function. It could as easily have been fortuitous or due to his general improvement.

It is our feeling that his recovery can also be attributed to the prompt recognition of the possibility of this diagnosis. The dispensary medical officer who first saw him recognized the possibility of a meningococcus infection and he was transferred to the hospital and directly to the contagious disease service within a very short time after he was first seen. The usual delays that occur when a patient has to be transferred from ward to ward were eliminated.

CONCLUSION

A case of meningococcus septicemia that had all the characteristics of a Waterhouse-Friderichsen syndrome is presented. This patient recovered. We feel his recovery was due to the use of plasma and adrenal cortex (both as extract and as desoxycorticosterone) to tide him over the period of adrenal insult; also to the use of sulfadiazine, and to the prompt recognition of the possibility of the diagnosis and the immediate institution of treatment.

CLINICAL CHEMISTRY

THERAPY OF MIGRAINE BY ELECTROLYTES AFFECTING THE BLOOD VOLUME

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HYPERTONIC solutions of various crystalloids[1] have been advocated in the treatment of migraine probably on the theory that the headache was caused by cerebral edema. Large and continuous oral doses of calcium salts[2-4] have also been advocated on the finding that some patients are benefited by this regime. Recently Greenhill and Freed[5] has used ammonium chloride to induce diuresis and thus prevent the syndrome of premenstrual distress which is characterized by edema, restlessness, and headache. The therapeutic regime for headache advocated by Goldzieher[6] involves the same therapeutic principle, namely, the mobilization of extracellular fluid.

Previous observations[7] have shown that migraine, menstrual migraine, and relaxation headache are associated with a relative decrease in the effective arterial blood volume. The purpose of this study was therefore to find an electrolyte or combination of electrolytes which would produce a temporary increase in blood volume.

Extensive studies on diuresis and water balance in dogs and rats[8] indicated that while both calcium and potassium were diuretic if given alone, when given together diuresis did not occur. The diuretic qualities were completely suppressed when they were administered in the proportion of 3 mols of potassium to one of calcium; a ratio which is approximately the same as that found in the blood plasma for these two ions. It was then shown that along with the suppression of diuresis an increase in the blood volume took place when a combination of these ions was given and that the increase was greater than when an equivalent amount of sodium salts were given. This combination of 3K-1Ca was then tried clinically in migraine.

EXPERIMENTAL

The hematocrit, as determined with Van Allen tubes using heparin as the anticoagulant, was used along with simultaneous serum protein determinations as an index of changes in the plasma volume. The serum proteins were determined gravimetrically after acetone precipitation. The effect of intravenous

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and oral Ca-K mixtures (Calcium lactate + KCl, $CaCl_2$ + KCl) on these two factors was compared with that of equi-molar mixtures of sodium salts (NaCl, Na citrate). (Table I and II.) The intravenous injection of the potassium mixture was carried out slowly and with care because of the pain of injection and the danger of cardiac depression.

TABLE I

COMPARISON OF THE HEMODILUTION OBTAINED BY 3K-1CA MIXTURES WITH THE NA SALT EFFECT. AFTER CONTROL SAMPLE THE MIXTURES WERE INJECTED SLOWLY. THE SECOND SAMPLE WAS TAKEN 20 TO 30 MINUTES LATER (AVE. 26 MIN.)

		CONTROL		26 MIN.	
		PROT. GM. %	HEM. %	PROT. GM. %	HEM. %
Mean of 17 subjects $CaCl_2$ — 0.6% KCl — 1.5%	10 c.c. I.V.	8.34	45.0	7.69	43.5
Mean of 6 subjects NaCl — 2.0%	10 c.c. I.V.	7.78	47.3	8.04	46.7

TABLE II

SAMPLES WERE TAKEN AT 30, 60, AND 120 MINUTES AFTER THE ORAL DOSE

	CONTROL		30 MIN.		60 MIN.		120 MIN.	
	PROT. GM. %	HEM. %	PROT. GM. %	HEM. %	PROT. GM. %	HEM. %	PROT. GM. %	HEM. %
Mean of 8 subjects 2.0 Gm. orally Ca Lactate-KCl Mixture	8.34	48.2	8.12	47.8	7.92	47.1	7.85	47.1
Mean of 5 subjects 2.0 Gm. Na Salts Orally	7.92	48.5	7.95	47.8	7.94	47.9	7.86	47.9

The data indicated regularly a greater hemodiluting effect with K-Ca combinations than could be accounted for by the control NaCl salt effect. It was then decided to try this therapy on an extensive scale in migraine. Accordingly, a questionnaire was prepared covering the salient points in the diagnosis of the syndrome, the concomitant symptoms, and important predisposing factors. This study was started in December, 1939, and over 200 patients have now been treated. Adequate histories and follow-up records were obtained on 150 of these 200 cases. The data are analyzed and presented for their value as a statistical study of the migraine syndrome itself as well as an evaluation of the electrolyte therapy employed.

RESULTS

The symptoms of diagnostic importance are summarized in Table III. While the familial history of migraine is about equal in both sexes, it is of interest that the incidence of visual symptoms is significantly greater in the male than in the female. That the incidence of gastric disturbance is greater than the incidence of visual symptoms was also noted by Von Storch.[9] He found also that the ratio of male to female patients is about 1 to 3. In our series it is 1 to 2.8

TABLE III

OCCURRENCE OF SYMPTOMS OF DIAGNOSTIC IMPORTANCE

	FEMALE PATIENTS 110		MALE PATIENTS 40	
1. Familial history	72	65%	26	65%
2. Visual symptoms*	36	33%	22	55%
3. Nausea and emesis	87	79%	28	70%
4. Hemicrania	41	37%	17	42%

*Severe photophobia included.

Data on minor factors in the migraine syndrome were obtained by direct questioning. The questions with the percentage of positive answers were as follows: Does excitement or nervous fatigue produce headache? (66%) Does going too long without food cause headache? (53%) Does relaxation (such as sleeping late) cause headache? (50%) Any increase in urine flow before or during headache? (33%) Any chocolate intolerance? (27%) Any carbohydrate intolerance? (19%) Does lack of customary coffee cause headache? (19%) Do attacks occur only at menstruation? (25% of females) Other questions regarding bulimia or irritability preceding the headache were also asked. Many patients volunteered answers, but they were not sufficiently concise to warrant tabulation and analysis. Seventeen out of eighteen of the patients who had become pregnant were free of headache during the second and third trimesters. Of these 17 patients several described a gradual return of their migraine in the postpartum months, while the majority had a return of their symptoms within one month. Two had severe migraine headaches several days postpartum. One patient was temporarily relieved by the development of thyrotoxicosis, one by catarrhal jaundice, and one by fever therapy. The results of therapy are tabulated in Table IV.

TABLE IV

DURATION OF MIGRAINE IN RELATION TO THERAPEUTIC RESULTS

THERAPEUTIC RESULTS	A		B		C		D	
Female (110)	23	21%	46	42%	30	27%	11	10%
Average years' duration	14		21		20		19	
Male (40)	13	32%	13	32%	10	25%	4	10%
Average years' duration	20		12.5		18		21	
Male and Female (150)	36 patients		59 patients		40 patients		15 patients	
	24%		39%		27%		10%	

A = Complete relief of migraine headaches.
B = Occasional headaches which now respond to acetyl salicylic acid.
C = Some effect but still require ergotamine tartrate therapy.
D = Therapy not effective.

The patients were treated by administration of the following formula:

Calcium lactate	308 Gm. = 1 Mol. equi.
Potassium chloride	225 Gm. = 3 Mol. equi.

These ingredients were mixed thoroughly in a mortar and packed into No. 0 capsules. When hand packed the capsule content was approximately gr. x (0.650 mg.). All of the patients were observed for a period of at least three months, and many of them have been observed for more than two years.

The most effective regime of therapy was found to be a gradual increase in dosage from gr. x of the mixture the first day to gr. xxx daily of the mixture at the end of one week. At the end of one month the patients were instructed to take only gr. x daily and to increase the dose by gr. xx every hour at the first sign of an impending headache. An increased, colorless urine flow was frequently found to precede the scotomata as a first sign of headache. It should be noted that the patients had well-established migraine histories and that success of the therapy was independent of the number of years the patients had had the disorder. As is usual in any salt therapy, relapses were sometimes encountered if the patient took large doses of the mixture over a period of several months. These were avoided by decreasing the dosage to a bare minimum at the end of one month. That these relapses were not due to the initial success which frequently accompanied any new medication was shown by the facts that: (1) substitution of NaCl and calcium lactate placebo capsules for the Ca-K mixture resulted in marked diminution in the therapeutic effect, and (2) a cessation of therapy precipitated attacks in many individuals and a return to the medication was again attended by at least a month's success.

TABLE V

THERAPEUTIC RESULTS IN 150 CASES ACCORDING TO SYMPTOMS AND PREDISPOSING FACTORS

	TOTAL CASES*	A	B	% A + B†	C	D	% C + D
1. Excitement	99	30	42	73%	22	5	27%
2. Starvation	79	24	31	70%	20	4	30%
3. Relaxation	75	21	36	76%	16	2	24%
4. Preceding diuresis	50	9	25	68%	14	2	32%
5. Chocolate intolerance	40	11	14	63%	14	1	38%
6. Carbohydrate intolerance	28	9	10	68%	9	0	32%
7. Caffeine withdrawal	29	8	14	76%	7	0	24%
8. Menstruation	27	5	12	63%	9	1	37%

*Number of patients with this symptom or finding.

†Percentage with A or B results (see Table IV). Seventeen out of 18 patients had their migraine relieved by pregnancy. One patient was migraine-free for a period of four months during an attack of catarrhal jaundice. One patient was relieved for a period of one year presumably by thyrotoxicosis since the headaches returned after the subtotal thyroidectomy.

Table V is an analysis of the data from the standpoint of predisposing factors to ascertain whether any one type of case responds more favorably than another. The relation of site of headache to the result of the therapy is interesting in that if the excellent and good results are pooled for the first three categories, the number of cases of right-sided headaches improved is much smaller than the percentage improvement for the left-sided and frontal headaches (38 per cent for right compared to 73 per cent and 76 per cent for the left and bilateral localizations, respectively). This finding may be of significance as an aid in prognosis and selecting patients for treatment.

The mixture of calcium lactate with potassium chloride greatly reduces the fleeting gastric pain frequently induced by the administration of crystalline potassium chloride alone. Less than 5 per cent of the patients noted any gastric sensation from the encapsulated mixture. Only one patient had to discontinue the treatment because of gastric irritation. Some patients ascribed an increased feeling of well-being and warmth of the extremities to the medication. A shortened menstrual cycle was noted in a few. Some patients reported a mild

but consistent cerebral stimulation and an increased ability to stand psychic trauma. No toxic symptoms were noted in the entire series. Several patients reported that a dull generalized headache occurred when too many capsules (10 to 20 per day) were taken. This is probably similar in etiology to the premenstrual headache encountered in some patients. It is relieved by drinking water to the point of diuresis or by the ingestion of caffeine. It is not relieved by acetylsalicylic acid.

TABLE VI

THE EFFECT OF THERAPY IN RELATIONSHIP TO THE INITIAL LOCALIZATION OF THE HEADACHE. 149* CASES

	A	B	C	D
Left frontal or temporal 26 = 17%	9 35%	10 38%	6 23%	1 4%
Right frontal or temporal 32 = 21%	5 16%	7 22%	13 41%	7 22%
Bifrontal or bitemporal 66 = 44%	17 26%	33 50%	13 20%	3 5%
Occipital 15 = 10%	4 27%	5 33%	3 20%	3 20%
Generalized 9 = 6%	2 22%	1 11%	5 56%	1 11%
Vertex 1		1		

*One case not localized.

DISCUSSION

The results of this form of therapy are in accord with the hypothesis set forth as a result of our previous studies,[7] namely, that migraine is perhaps due to uncompensated fluctuations in the effective arterial blood volume. The hypothesis accounts for many of the vagaries of the migraine syndrome, namely, the onset at puberty and frequent relief at the menopause (correlating with the onset and waning of cyclic activity of the sex hormones with their salt and water retaining properties, the increased incidence in the female over the male due to the greater salt and water retaining power of estrogens over androgens[10] and relief of migraine by pregnancy where a normal concomitant increase in the blood volume obtains. Actual proof of this hypothesis must await experimental methods whereby the effective arterial blood volume can be measured and correlated with cardiac output and peripheral vascular tone. It must also be assumed that an underlying anatomical or physiological familial defect exists which allows vascular spasm or dilation with changes in the effective arterial blood volume.

The suggested salt mixture is certainly more effective than are calcium, potassium or sodium salts when given alone. It has the disadvantage of all salt therapy that some patients become tolerant after several months of use. No attempt was made to reduce NaCl intake during the period of treatment. Also the patients were not restricted in their use (when necessary of acetylsalicylic acid, phenacetin, or ergotamine tartrate). No toxic reactions were observed in the use of these common drugs with the salt mixture. The salt mixture should be given at the first sign of a headache since it can only be given orally. The patients who consistently awaken with a migraine syndrome so severe that

vomiting prevents oral retention of the capsules are, of course, not benefited by therapy unless it is taken prophylactically at bedtime.

Based on the findings of Graham[11] and also Atkinson[12] that nicotinic acid is of value in functional disorders, we have recently been treating a series of patients with the calcium-potassium mixture to which 1 per cent nicotinamide has been added. The preliminary results seem even more promising than the use of the salt mixture alone.

SUMMARY

The results of therapy of migraine over a period of three years with a mixture of calcium lactate and potassium chloride are reported. Of 150 patients 24 per cent had excellent results, 39 per cent good results, 27 per cent fair results (still require ergotamine tartrate) and in 10 per cent the therapy was noneffective. The detailed study revealed that spontaneous diuresis may precede the scotoma as the initial sign of an impending attack. Relaxation or "let down" in activity may be a precipitating factor in the migraine syndrome. In this series a better therapeutic response was obtained in patients with left-sided headaches than in those with right-sided headaches.

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CARDIOTOXIC SUBSTANCES IN THE BLOOD AND HEART MUSCLE IN UREMIA (THEIR NATURE AND ACTION)*

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CARDIAC failure is a common event in the terminal stages of renal insufficiency and uremia. While its manifestations are often overshadowed by other uremic signs, such as central nervous disturbances, vomiting, hemorrhages, etc., it can be considered as the ultimate cause of the fatal outcome in the majority of cases. Dyspnea and attacks of pulmonary edema are particularly conspicuous (Gouley; Luisada; and others) and also peripheral edema appears frequently in advanced stages. Pulsus alternans (Heitz; Chalier and Contamin), tachycardia, and bradycardia are often observed.

Marked alterations of the electrocardiogram, mainly of the "anoxic" type (flattening or inversion of the T waves, depression of the S-T interval) are frequently present in uremia (Wood and White; Becher; Gouley; Agnoli and Bussa).

Severe degenerative myocardial lesions have been found to be a characteristic of the uremic heart (Merklen and Rabé; Külbs; Lüscher; Gouley; Solomon, Roberts, and Lisa) and a diminished myocardial creatine concentration was observed in the failing heart muscle of uremic persons by Gross and Sandberg.

All investigators agree in the assumption of an unknown "toxin" as being the cause of myocardial damage and failure in uremia. The serum of dogs with experimental uremia and the serum of uremic patients contain unknown substances which exert a "digitalis-like" toxic effect on the isolated frog heart (Mason, Resnik, Minot, Rainey, Pilcher, and Harrison), and cause anoxic changes of the electrocardiogram of the guinea pig, according to Agnoli and Bussa who studied the effects of intraperitoneal or intravenous injection of crude serum or of serum dialysates.

The possibility that these substances may belong to the group of phenols was mentioned by Mason and his associates who observed a cardio-inhibitory effect of phenol and paracresol on the isolated frog heart. Retention of phenols in the blood as a regular feature of uremia was reported by Becher, by Marcolongo, and by Dickes.

Another characteristic anomaly of blood composition in uremia was described by the writer (Raab[1]), namely, the presence of excess amounts of substances with a catechol nucleus (benzene ring with two free hydroxyl groups in ortho-position). Such substances are pyrocatechol and a number of catechol

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compounds, including adrenalin, sympathin, adrenalone, dihydroxyphenylalanine, epinine, leucoadrenochrome, and others. The colorimetric method used for the quantitative determination of these substances in blood and tissues (Raab[1, 2, 3]) includes also ascorbic acid by virtue of its two adjacent hydroxyl groups on a lactone ring but, owing to its weak color intensity, ascorbic acid forms only a fraction of the colorimetric results and, in general, does not constitute a seriously disturbing factor.

The term "AC" will henceforth be used, standing for "adsorbable chromogens" (not "adreno-cortical" compounds, as in some previous publications when the colorimetric results were erroneously interpreted as being due to a combination of adrenalin with cortical steroids).

By far the highest AC concentrations were encountered in the adrenal medulla, and extensive experimental series have shown that the AC concentration in blood and tissues is largely dependent on adrenal secretion and probably also on peripheral sympathin production (Raab,[1, 2, 3] Cannon and Lissák). It appears reasonable, therefore, to consider the adrenal medulla and the peripheral sympathetic "adrenergic" nerve endings as the source of the bulk of AC material, consisting of adrenalin and related substances, which is present in blood and tissues in physiologic as well as pathologic conditions.

The heart muscle, although containing rather low AC concentrations under standard conditions, is characterized by an outstanding tendency to accumulate both artificially administered catechol compounds and spontaneously secreted AC substances (Raab[1, 4]). Accumulation of excess AC concentrations in the heart muscle beyond a certain critical level was found to be fatal in experimental animals, leading to cardiac failure and death (Raab[1]). Also in the majority of patients who had died from heart failure, the total AC concentration or the concentration of adrenalin (sympathin) proper in the myocardium was found above the upper limit of normal (Raab[1]).

The striking toxic effects of excess amounts of the most powerful catechol compound, adrenalin, on the electrocardiogram, and on metabolism, structure, and function of the myocardium are well known and were recently reviewed by the author (Raab[1]). Prolonged action of adrenalin upon the heart increases the sensitivity to inhibitory stimuli of the vagus nerve (Langecker; Sollmann and Barlow[1]) and adrenalin concentrations stronger than $1:10^6$ exert a directly inhibitory toxic effect upon the heart muscle (Sollmann and Barlow[2]). Other catechol compounds, such as adrenalone, produce effects similar to those of adrenalin on the heart and, although weaker than adrenalin (Tainter; Tani), seem to intensify the efficiency of the latter (Kato and Aibara). Catechol proper (pyrocatechol) acts as a cardiac depressant (Tainter).

In view of the above-mentioned facts, the behavior of AC substances in the blood and in the heart muscle of uremic patients was studied. It was correlated with the electrocardiogram and the clinical picture, and comparative experiments with uremic serum and with various catechols and phenols were carried out on the isolated frog heart and on the intact rabbit.

TABLE I

CASE	AGE	SEX	DATE*	N.P.N. MG. PER 100 C.C.	BLOOD CREATININE MG. PER 100 C.C.	URINE		RED BLOOD COUNT (MILLION PER CU. MM.)	MENTAL CONDITION	ACT† COL. UN. PER C.C.	D.S.R.‡	BLOOD PRESSURE	CARDIAC SYMPTOMS	ORIGIN OF UREMIA	COMPLICATIONS
						SPECIFIC GRAVITY	ALBUMIN								
1	57	♂	5/ 4/40	260	4	1008-10	Trace	3.6	Normal	562	1.01	160/ 90	Dyspnea, palpitations, pulmonary congestion	Congenital polycystic kidney	
2	45	♀	4/22/42	80	–	Almost anuric	+++	–	Drowsy	156	1.00	190/102	Dyspnea, marked edemas	Acute glomerulonephritis	Hematuria
			4/25/42	100	–				Drowsy	270	1.13	154/ 88	Dyspnea, marked edemas		
3	54	♂	7/13/42	125	9	Anuric	–			152	1.18	130/ 70	None	Acute nephritis (mercury poisoning)	Dermatitis
			7/24/42	180	10	1007-20	50%	5.0	Normal	257	1.00	146/ 92	Sl. ankle edema		
			8/ 4/42	72	–		Trace			85	1.21	–	None		
4	16	♀	12/ 4/40	333	27	1008-12	7-19%	1.9	Drowsy	420	1.00	135/ 85	None	Chronic glomerulonephritis	Sinusitis
5	8	♀	3/ 5/41	100	8	1005-15	15-30%	3.1	Sl. drowsy	362	1.00	162/ 90	Marked edemas, dyspnea	Chronic glomerulonephritis	Epistaxis
6	39	♂	12/ 6/39	104	–	1013-16	5-12%	4.0	Normal	304	–	230/140	Sl. dyspnea, cyanosis, palpitations	Chronic glomerulonephritis	Silicosis
7	20	♂	12/ 2/40	185	9	1004-10	Tr.-13%	3.6	Sl. drowsy	283	1.07	165/ 90	None (edema of face)	Chronic glomerulonephritis (lead?)	
8	64	♀	5/ 6/43	150	5	1014-15	12-25%	3.8	Drowsy	310	1.10	222/104	Dyspnea, marked edemas, tachycardia, hemorrhagic cough	Chronic glomerulonephritis	Subcutaneous hemorrhages
9	26	♀	6/ 7/43	123	6	1005-07	Trace	3.1	Drowsy	475	1.03	224/120	Dyspnea, palpitations	Chronic glomerulonephritis	
10	17	♂	11/25/42	228	5	1008-12	Tr.-2%	4.2	Sl. drowsy	176	1.10	126/ 84	Slight dyspnea	Chronic glomerulonephritis	Foot injury
11	11	♀	11/25/42	100	4	1010-11	Tr.-1%	4.1	Normal	146	1.11	130/ 90	None	Chronic glomerulonephritis	

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TABLE I—CONT'D

CASE	AGE	SEX	DATE*	N.P.N. MG. PER 100 C.C.	BLOOD CREATININE MG. PER 100 C.C.	URINE		RED BLOOD COUNT (MILLION PER CU. MM.)	MENTAL CONDITION	ACT. COL. TN. PER C.C.	D.S.E.†	BLOOD PRESSURE	CARDIAC SYMPTOMS	ORIGIN OF UREMIA	COMPLICATIONS
						SPECIFIC GRAVITY	ALBUMIN								
12	26	♀	1/22/43	220	28					290	1.02	154/115	Moderate dyspnea and edemas, hemorrhagic cough	Pyelonephritis	
			6/ 2/43	–	–	1009-16	6-13%	1.9	Drowsy	381	1.03	150/120	Moderate dyspnea and edemas, hemorrhagic cough		
			3/ 8/43	275	6					310	1.14	152/116	Moderate dyspnea and edemas, hemorrhagic cough		
			3/27/43	229	–					620	1.14	–	Moderate dyspnea and edemas, hemorrhagic cough		
13	53	♀	11/25/42	320	–	1012-16	Trace	3.8	Deep coma	660	1.02	132/ 90	Tachycardia	Pyelonephritis	Pneumonia, epilepsy
14	33	♀	4/13/43	153	8	1009-13	14%	3.0	Sl. drowsy	543	1.03	172/122	Dyspnea, tachycardia, ankle edema	Pyelonephritis (after pregnancy)	Subcutaneous hemorrhages
15	38	♀	9/10/41	680	21	1006-10	2-4%	3.8	Excited	362	1.10	160/100	Edemas, moderate dyspnea	Obstructive pyelonephritis	Ca. of uterus (pelv. metast.)
16	60	♀	8/ 6/42	196	2	1010-25	Tr.-27%	3.8	Deep coma	315	1.14	172/ 80	Cheyne-Stokes respiration, edemas	Obstructive pyelonephritis	Uterus prolapse, diabetes
17	50	♂	4/27/40	270	–	1009-11	18%	3.0	Irrational	302	0.85	190/ 98	Orthopnea, marked edemas	Obstructive pyelonephritis	Prostatic hypertrophy
18	76	♂	11/ 8/41	133	–	1007	24%	1.7	Drowsy	265	–	130/ 80	Dyspnea, edemas	Obstructive pyelonephritis	Prostatic hypertrophy
19	64	♂	1/23/41	364	17	1008	8%	2.0	Coma	–	–	138/ 78	Dyspnea, edemas	Obstructive pyelonephritis	Prostatic hypertrophy
20	72	♂	4/27/43	142	9	1009-16	Trace	4.0	Irrational	–	–	184/100	Cheyne-Stokes respiration, hydrothorax	Obstructive pyelonephritis	Carcinoma of prostate

21	52	♂	4/ 5/40	171	6					362	0.94	235/141	Orthopnea	Nephrosclerosis	Epistaxis
			4/ 8/40			1008-10	3%	3.2	Drowsy	377	–	242/130	Edemas		
			4/15/40§							257	–	230/126	Edemas		
			4/22/40							556	1.05	170/ 85	Pulmonary edema		
22	39	♂	7/11/41	267	9	1010-12	2-8%	3.4	Drowsy	367	1.00	238/144	Dyspnea, hemorrhagic cough, edemas, palpitations	Nephrosclerosis	Epistaxis
23	46	♀	8/27/42	132	–	1005-18	Tr.-9%	4.1	Coma	260	–	188/120	Orthopnea, edemas, palpitations, tachycardia	Nephrosclerosis	Epistaxis, pneumonia
24	53	♂	4/ 7/40	84	5	1008-12	Neg.	4.5	Normal	169	–	230/140	Dyspnea, hemorrhagic cough	Nephrosclerosis	
			11/19/40	217		1009-11	Tr.-3%	4.2	Normal	217	–	220/110	Dyspnea, hemorrhagic cough		
25	58	♂	3/ 1/42	100	–		Trace	4.8	Drowsy	–	–	225/130	Cheyne-Stokes respiration, pulmonary edema	Nephrosclerosis	
26	25	♂	9/ 8/39	55						558	–	190/140	Cheyne-Stokes respiration, edemas, tachycardia	Nephrosclerosis (paraganglioma?)	Right adrenal denervation 3 months previously
			9/11/39‖	130		1008-11	Trace	3.4	Excited	257	–	220/140	Cheyne-Stokes respiration, edemas, tachycardia		
			9/16/39	133	16					290	–	230/170	Cheyne-Stokes respiration, edemas, tachycardia		
27	34	♂	6/26/43	172	–	1003-11	2-14%	3.1	Sl. drowsy	305	1.03	215/130	Marked edemas, dyspnea	Nephrosclerosis	
28	65	♀	11/ 2/42	112	–	1016	1%	4.4	Deep coma	460	0.98	187/118	Tachycardia	Perforation of bladder, deviation of urine into peritoneal cavity	Pseudomyxoma of peritoneum

*Concerns the day on which blood AC was determined. In several instances the N.P.N., etc., had been determined one or a few days previously.
†Stands for "adsorbable chromogens" (see page 716).
‡Stands for "denominator of specific ratio" (see page 720).
§Three days after a series of x-ray irradiation of the adrenal glands.
‖After a series of x-ray irradiation of the adrenal glands.

METHODS

1. AC in blood and heart muscles was determined by the modified method of Shaw, as described by the writer (Raab[1]). The results are expressed in color units per cubic centimeter or gram, each unit corresponding to the color intensity of 10^{-6} mg. of adrenalin, which was used for standard comparison. The "d.s.r." (denominator of specific ratio) permits a rough evaluation of the qualitative composition of the bulk of AC material found. A "d.s.r." of 2 or more indicates the prevailing or exclusive presence of adrenalin and/or sympathin; lower d.s.r.'s indicate the presence of other related catechol compounds, such as adrenalone, dihydroxyphenylalanine, leucoadrenochrome, etc. An occasional d.s.r. below 1.0 can be assumed to be due to a relatively large amount of ascorbic acid.

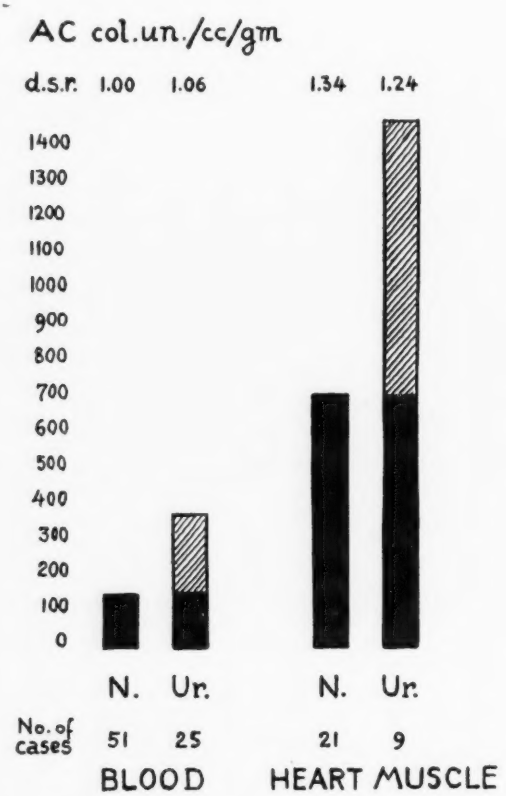

Fig. 1.—Average concentrations of catechol compounds (AC = adsorbable chromogens) in the blood and heart muscle of normal and uremic persons.

2. The frog heart experiments were carried out with hearts of *Rana pipiens*, prepared according to the Straub method, suspended in a moist chamber in an oxygen atmosphere and filled with Ringer's solution which was replaced by fresh serum or various experimental solutions.

3. Alcoholic serum extracts were prepared as follows: 50 c.c. of serum (mixed with an equal amount of 95 per cent alcohol for disinfection) were dried forty-eight hours in a flat plate at a temperature of 37 to 38° C. The

dried serum was finely pulverized in a mortar and extracted two hours in a Soxhlet apparatus with 250 c.c. of absolute alcohol. The alcohol was then evaporated with suction over a hot water bath and the extract cautiously boiled down to a volume of 2½ c.c. This brownish lipid containing residue was kept in small vials in the dark.

4. The rabbit electrocardiograms were taken with a Siemens Atlanco apparatus under nembutal anesthesia.

CATECHOL COMPOUNDS IN THE BLOOD, AND CARDIAC MANIFESTATIONS IN UREMIC PATIENTS

Twenty-eight uremic patients, each with a N.P.N. concentration of at least temporarily 100 mg. per 100 c.c. or more, were examined (Table I). In 19 of these patients the blood creatinine was determined. It was 4 mg. per 100 c.c. or more in 18 cases.

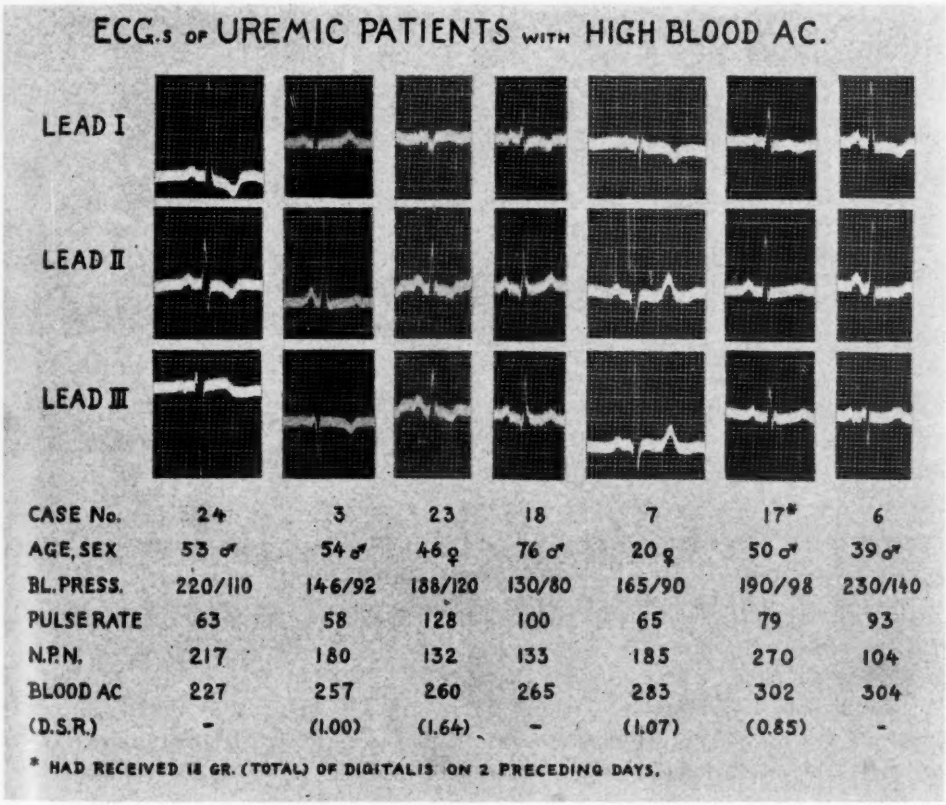
ECG.s OF UREMIC PATIENTS WITH HIGH BLOOD AC.
LEAD I
LEAD II
LEAD III
CASE No. 24 3 23 18 7 17* 6
AGE, SEX 53 ♂ 54 ♂ 46 ♀ 76 ♂ 20 ♀ 50 ♂ 39 ♂
BL. PRESS. 220/110 146/92 188/120 130/80 165/90 190/98 230/140
PULSE RATE 63 58 128 100 65 79 93
N.P.N. 217 180 132 133 185 270 104
BLOOD AC 227 257 260 265 283 302 304
(D.S.R.) - (1.00) (1.64) - (1.07) (0.85) -
* HAD RECEIVED 18 GR. (TOTAL) OF DIGITALIS ON 2 PRECEDING DAYS.

Fig. 2.

In all but three cases (Nos. 10, 11, and 24) the blood AC values were abnormally high (Fig. 1), the normal average (51 cases) being 156 col. un./c.c. ranged between 0.85 and 1.21, thus indicating the prevailing presence of and the highest level of normal being 222 col.un./c.c. (Raab).[7] The d.s.r. catechol compounds not identical with either adrenalin or sympathin, a condition analogous to the normal,

Signs of cardiac failure were present in the majority of all cases: dyspnea (partly Cheyne-Stokes respiration) (21 cases); pulmonary edema and/or cough with bloody sputum (7 cases); peripheral edemas (16 cases); tachycardia and/or palpitations (10 cases); bradycardia (3 cases). Twenty-one cases were hypertensive. Twenty-three cases were mentally disturbed, drowsy, irrational, or comatose.

Death occurred within a few hours to a few weeks after examination in 19 cases; the further fate of 7 cases is unknown to the writer; two (Cases 3 and 27) improved very considerably and have survived for several months so far, despite high N.P.N., creatinine, and AC values at the time of examination.

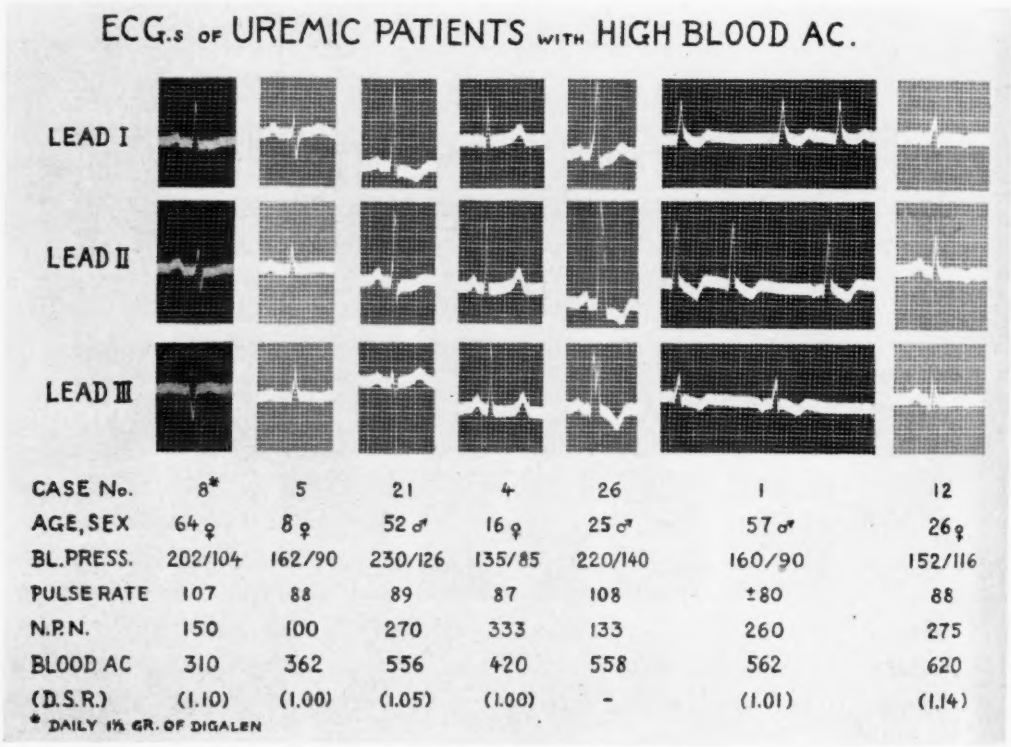

Fig. 3.

In 25 cases electrocardiograms were taken (Figs. 2-4). In 12 the axis position was normal, in 9 there was a left, in 4 a right, axis deviation. Only 5 were free of alterations of the "anoxic type" (Cases 3, 4, 10, 11, 14, and 16). In 20 cases there was flattening or inversion of the T waves (of T_1 alone seven times; of T_1 and T_2 four times; of T_2 and T_3 four times; of T_1, T_2, and T_3 five times). The S-T interval was depressed in 9 cases (S-T_1 twice; S-T_1 and S-T_2 three times; S-T_2 alone three times; S-T_1, S-T_2, and S-T_3 once). In 2 cases (Nos. 15 and 18) S-T_2 and S-T_3 were slightly elevated. The P-R interval was 0.20 or more in 4 cases (Nos. 5, 8, 20, and 22) and 0.12 in one case (No. 23). Digitalis medication was being used at the time of the examination in 4 of the cases with anoxic electrocardiograms (Nos. 2, 8, 9, and 17).

No definite relationship between the degree of AC elevation above normal and the degree of electrocardiographic changes could be established but it appears significant that the only 2 cases with clearly normal AC values (Nos. 10, and 11) had also normal electrocardiograms, and that in 3 cases in which the electrocardiogram was examined repeatedly (Nos. 3, 12, and 24) the anomalies of the T wave appeared or became markedly accentuated as the blood AC level rose.

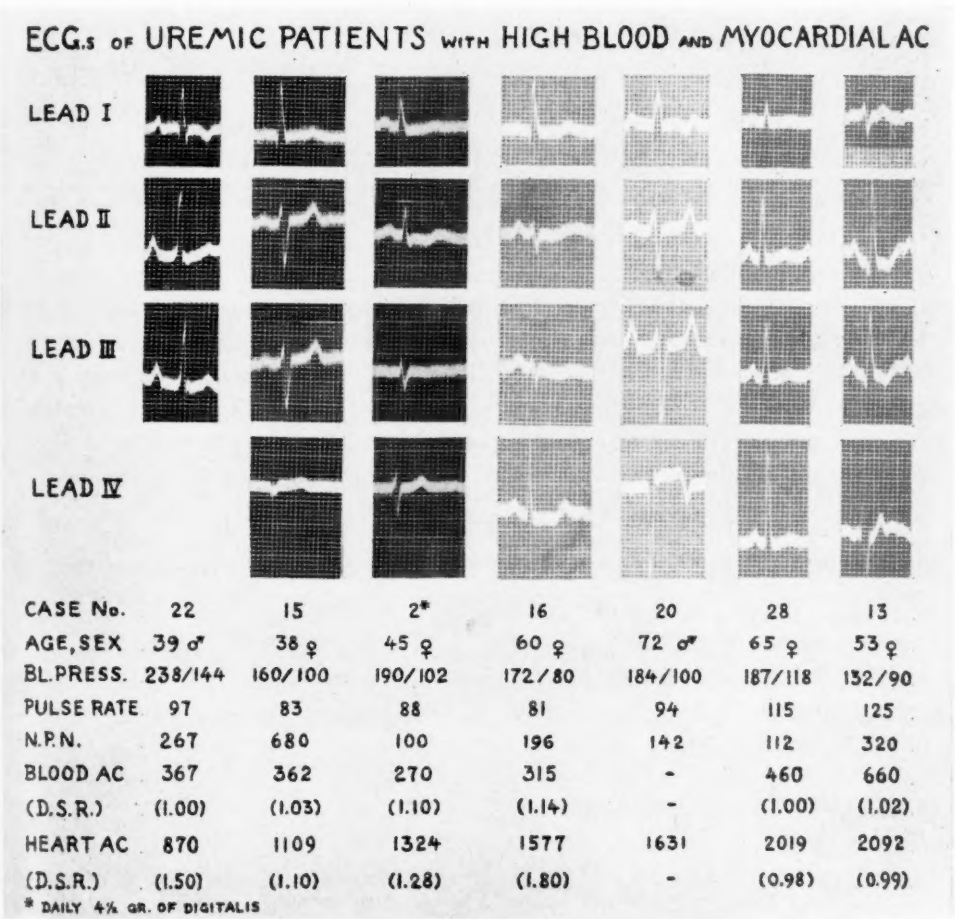

Fig. 4.

AC IN THE HEART MUSCLE OF UREMIC PATIENTS AND OF EXPERIMENTAL RATS

In 9 of the uremic cases in which an autopsy* was performed the heart muscle was examined for its AC content (Table II, Fig. 1). In 21 normal (nonfailing) human hearts previously examined, the average AC concentration per gram of myocardial tissue (area near the angle of the descending left coronary branch) was 698 col.un./Gm. Assuming about 1,000 col.un./Gm. as

*The autopsy findings were furnished by the Department of Pathology, University of Vermont.

TABLE II

ABNORMALLY HIGH AC CONCENTRATIONS IN THE UREMIC HEART MUSCLE (THE NORMAL AVERAGE IS 698 COL.UN./GM.)

CASE	WEIGHT OF HEART (GM.)	AC OF HEART MUSCLE (COL. UN./GM.)	D.S.R.	MICROSCOPIC FINDINGS
2	300	1,324	1.28	Negative*
13	250	2,092	0.99	Not examined
15	500	1,109	1.10	Negative
16	350	1,577	1.80	Not examined
19	–	1,463	0.98	–
20	500	1,631	–	Marked fragmentation and light staining of fibers, edema
22	500	870	1.50	Generalized fibrosis, cells poorly staining, striations not distinct
25	700	1,242	1.34	Not examined
28	240	2,019	0.98	Not examined

*Macroscopically the myocardium appeared pale and soft, and the right coronary artery was almost occluded.

the upper limit of normal, it can be stated that in 8 out of 9 uremic cases the myocardial AC concentration was markedly elevated above normal, and in the one remaining case (No. 22) it was above average and not far below the upper limit of normal. The d.s.r. varied, as in normal hearts, between 0.98 and 1.80, thus indicating a prevalence of catechol compounds not identical with either adrenalin or sympathin (except in the one instance with a d.s.r. of 1.80 in which almost pure adrenalin or sympathin seemed to be present).

TABLE III

DEPOSITION OF INJECTED CATECHOL COMPOUNDS IN THE HEART MUSCLE OF THE RAT

SUBSTANCE INJECTED	RAT	DOSE PER GM. OF BODY WEIGHT (MG.)	INTERVAL BETWEEN INJECTION AND DEATH (MIN.)	MODE OF DEATH	AC (COL.-UN./GM.)	D.S.R.
Pyrocatechol (d.s.r. 1.00)	1	0.05	20	Killed	1,561	1.00
	2	0.1	20	Killed	3,035	1.10
	3	0.5	5	Spontaneous	18,301	0.91
	4	1.7	2½	Spontaneous	38,759	1.00
Adrenalone (d.s.r. 1.06)	5	0.25	20	Killed	3,654	1.12
	6	0.25	20	Killed	4,040	1.24
	7	0.25	20	Killed	3,776	1.12
	8	0.75	20	Killed	6,818	1.00
	9	1.50	19	Spontaneous	11,167	1.01
Dihydroxyphenyl-alanine (d.s.r. 1.06)	10	0.15	20	Killed	4,962	1.07
	11	0.75	20	Killed	14,596	1.03

In order to gather some information as to which catechol compounds beside adrenalin (which had been studied previously in this respect, Raab[1]) are absorbed by and deposited in the heart muscle, large doses of pyrocatechol, adrenalone, and dihydroxyphenylalanine were injected subcutaneously into white rats, and the myocardial AC concentration was subsequently examined (Table III). The results indicate clearly that the heart muscle possesses an

outstanding tendency to absorb and accumulate not only adrenalin, as demonstrated elsewhere, but also other related catechol compounds, their d.s.r. remaining practically unchanged. The increase of AC in the heart muscle within a limited time was roughly proportionate to the injected doses:

	Pyrocatechol	Adrenalone	Dihydroxyphenylalanine
Dosages:	1 : 2 : 10 : 34	1 : 3 : 6	1 : 5
Increase in heart AC:	1 : 2.5 : 18 : 38	1 : 2 : 3.3	1 : 3

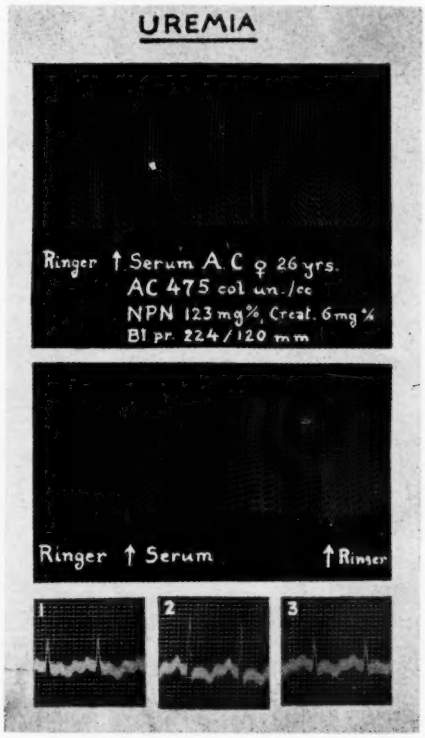

Fig. 5.—Effect of uremic serum on the isolated frog heart. Type 1: Increase of amplitude. The electrocardiogram is that of the patient whose serum was used in this experiment (Case 9).

EFFECT OF UREMIC SERUM AND OF CATECHOL COMPOUNDS UPON THE ISOLATED FROG HEART

The effect of fresh uremic sera upon the isolated frog heart was compared with that of the fresh sera of 20 nonuremic persons, 9 of them hypertensive and 11 nonhypertensive. All nonuremic sera caused a slight to moderate increase in amplitude, ranging from +7 per cent to +28 per cent in the nonhypertensive and from +6 per cent to +38 per cent in the hypertensive cases. Significant alterations of the rhythm were not encountered. The effect of uremic sera, on the other hand, was essentially of three types: (1) A very marked increase in amplitude, ranging from +48 per cent (Fig. 5, Case 9) to +1250 per cent (Fig. 6, Case 8) and even resuscitation of the nonbeating heart (Fig. 6) to extremely vigorous contractions. This effect was produced by the sera of the

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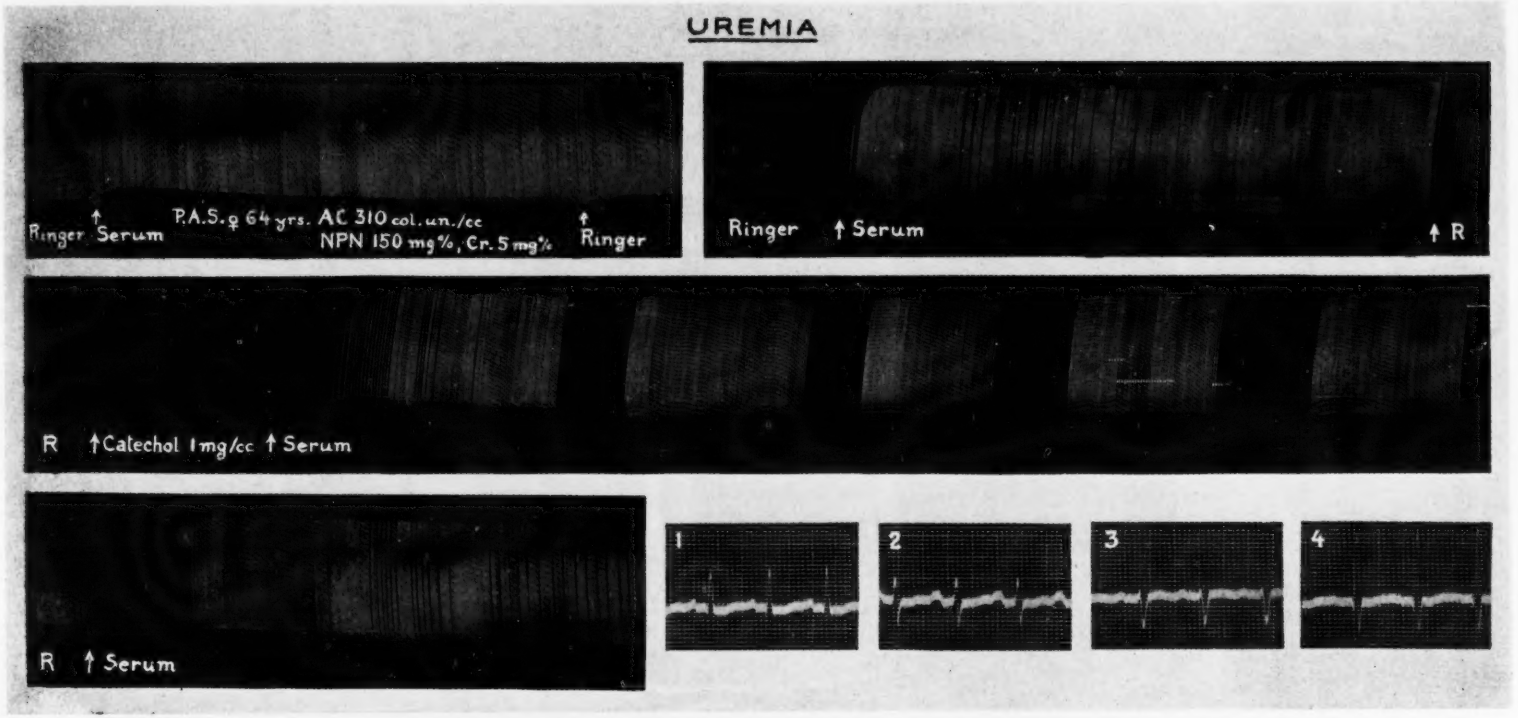

Fig. 6.—Effect of uremic serum on the isolated frog heart. Type 3: Combination of increased amplitude with periods of cardiac standstill. Electrocardiogram of the patient whose serum was used in this experiment (Case 8).

uremic cases (Nos. 8, 9, and 27). It cannot be attributed to preceding digitalis medication since it was present also in Case 27 in which no digitalis had been given. (2) Marked bradycardia and periods of cardiac standstill without any significant change of amplitude (Figs. 7 and 8, Cases 12 and 14). (3) A combination of increased amplitudes and periodic standstills (Fig. 6, Case 8). Replacement of the uremic serum by Ringer's solution abolished the bradycardia almost immediately but the amplitude-increasing effect persisted somewhat longer.

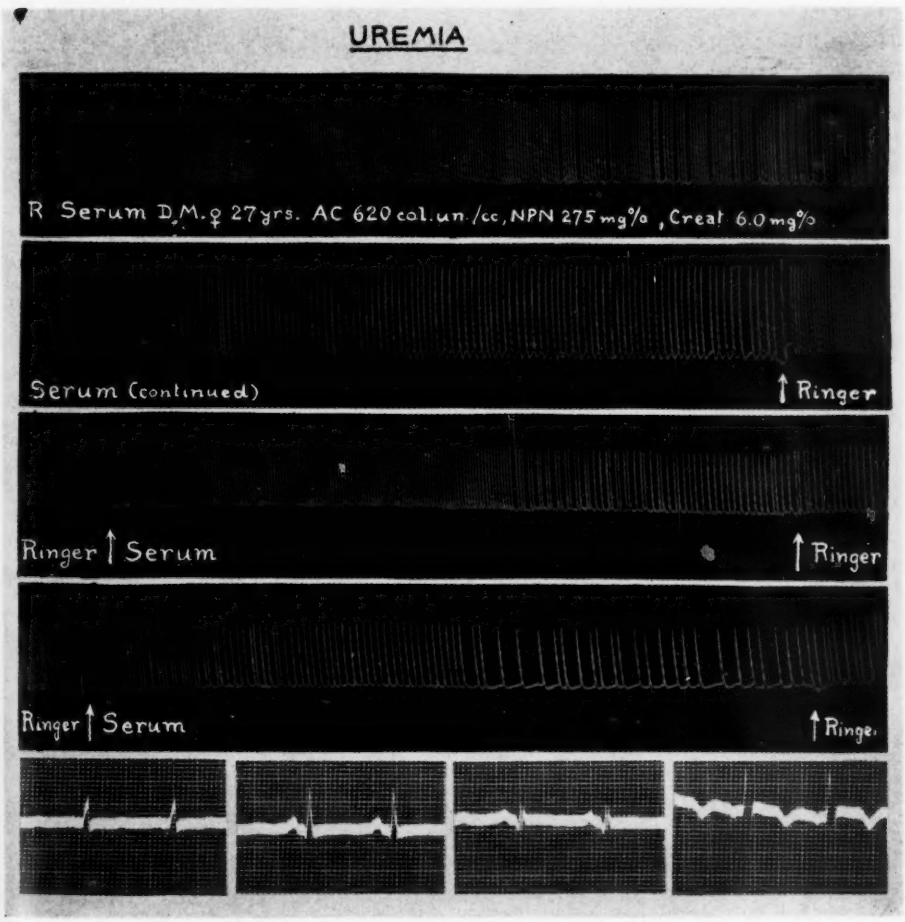

Fig. 7.—Effect of uremic serum on the frog heart. Type 2 a: Gradually increasing bradycardia without significant increase of amplitude. The electrocardiogram is that of the patient whose serum was used (Case 12).

Attempts were made to reproduce some of the above-named effects of the uremic serum upon the isolated frog heart by introducing various catechol compounds other than adrenalin dissolved in Ringer's solution. Increases of the amplitude, although only of a very slight degree, were produced by adrenalone and dihydroxyphenylalanine, while pyrocatechol (catechol proper) diminished the amplitude and caused persistent cardiac standstill (Table IV and Fig. 9).

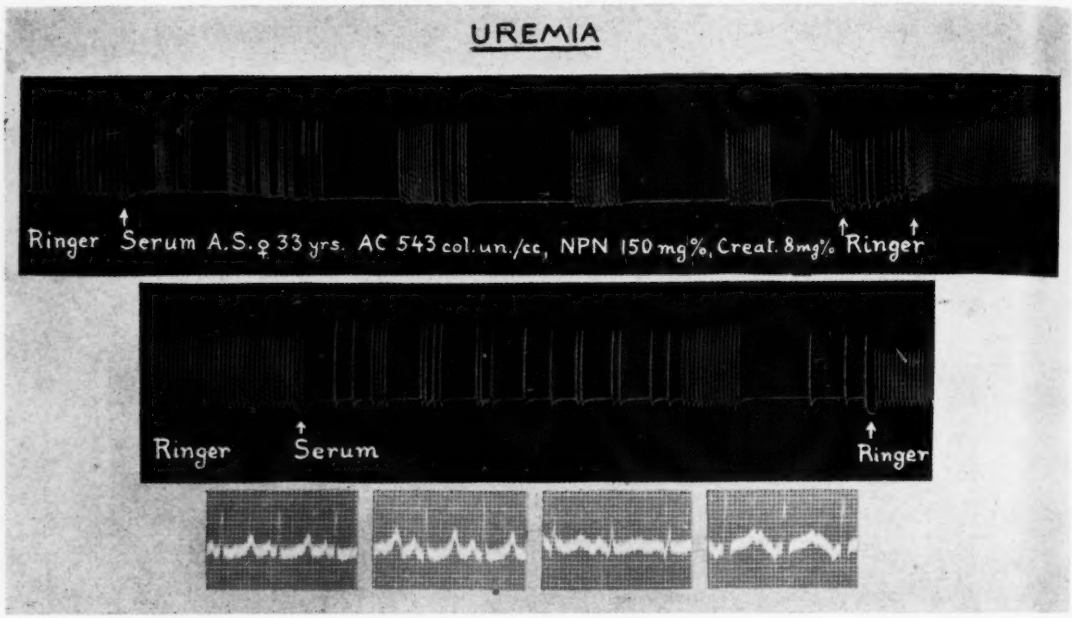

Fig. 8.—Effect of uremic serum on the frog heart. Type 2b: Bradycardia and periods of cardiac standstill without significant increase of amplitude (Case 14). The effect is immediately abolished by Ringer solution.

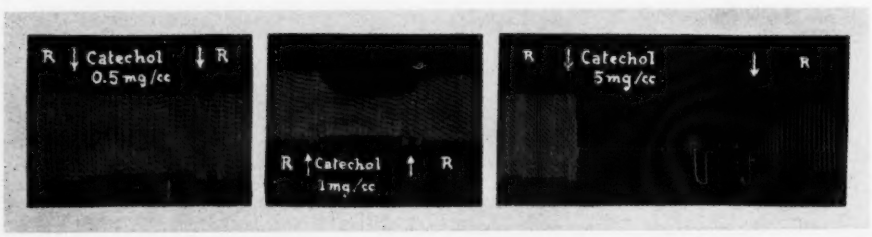

Fig. 9.—Effect of various catechol (pyrocatechol) concentrations on the isolated frog heart.

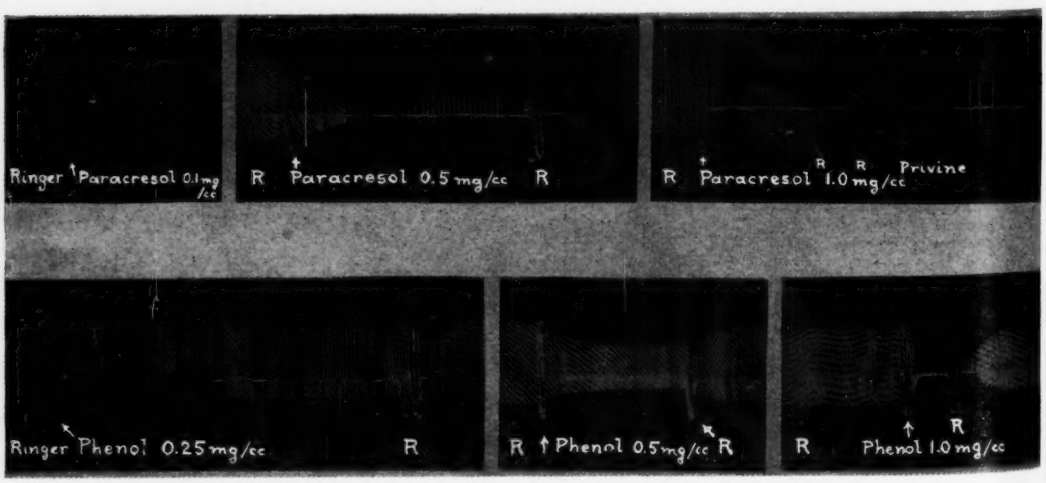
Ringer Paracresol 0.1 mg/cc
R Paracresol 0.5 mg/cc R
R Paracresol 1.0 mg/cc Privine
Ringer Phenol 0.25 mg/cc R
R Phenol 0.5 mg/cc R
R Phenol 1.0 mg/cc

Fig. 10.—Effect of various concentrations of phenols on the isolated frog heart.

TABLE IV

EFFECT OF CATECHOLS AND PHENOLS ON THE AMPLITUDE OF THE ISOLATED FROG HEART

SUBSTANCE	CHANGE OF AMPLITUDE THROUGH MG. OF SUBSTANCE PER C.C. OF SOLUTION							
	0.02	0.1	0.25	0.4	0.5	1.0	2.0	5.0
Adrenalone	+7%	+9%	–	–	+19%	–	–	–
Dihydroxyphenylalanine	–	–	–	+11%	–	–	+19%	–
Pyrocatechol	–	–	–6%	–	–16%	–41%	–	–100%
Phenol	–	–	–8%	–	–44%	–100%	–	–
Paracresol	–	–10%	–	–	–67%	–100%	–	–

Carbolic acid (phenol) and paracresol as representatives of the phenol compounds, which are also regularly increased in the uremic serum, were likewise examined regarding their effect upon the frog heart. According to dosage they caused diminution of amplitude, bradycardia, and/or periodic or lasting cardiac standstill (Table III, Fig. 10).

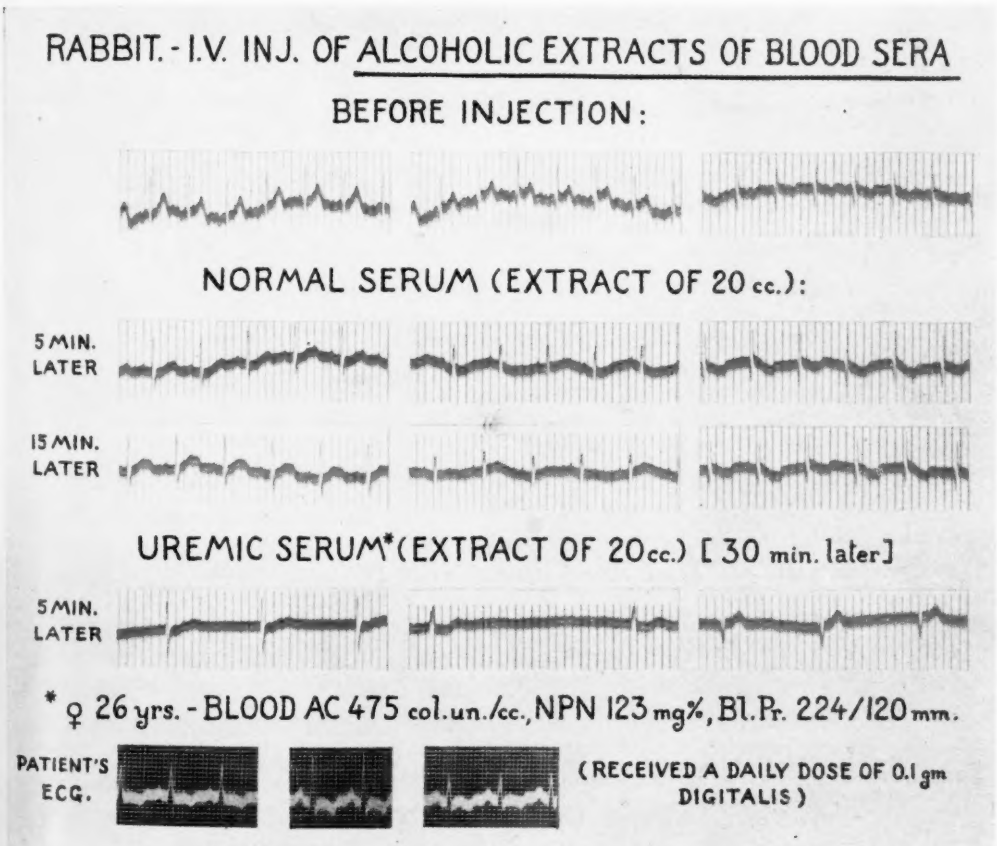
RABBIT. - I.V. INJ. OF ALCOHOLIC EXTRACTS OF BLOOD SERA
BEFORE INJECTION:
NORMAL SERUM (EXTRACT OF 20 cc.):
5 MIN. LATER
15 MIN. LATER
UREMIC SERUM* (EXTRACT OF 20 cc.) [30 min. later]
5 MIN. LATER
* ♀ 26 yrs. - BLOOD AC 475 col.un./cc., NPN 123 mg%, Bl.Pr. 224/120 mm.
PATIENT'S ECG.
(RECEIVED A DAILY DOSE OF 0.1 gm DIGITALIS)

Fig. 11.—Effect of normal and of uremic serum upon the rabbit's electrocardiogram. (Intravenous injection of alcoholic serum extracts.) Death followed three minutes after the last electrocardiogram.

EFFECT OF ALCOHOLIC EXTRACTS OF UREMIC SERUM AND OF CATECHOL COMPOUNDS UPON THE RABBIT'S ELECTROCARDIOGRAM

In order to test the effect of the cardiotoxic constituents of uremic serum upon the rabbit's electrocardiogram, it was necessary to eliminate all protein

material. Catechol compounds which are otherwise insoluble in organic solvents can be extracted from tissues in the presence of lecithin with chloroform and alcohol (Kendall; Raab[3]). The quantitative recovery of the total AC from serum by alcoholic Soxhlet extraction had been ascertained before through colorimetric analysis (Raab[4]).

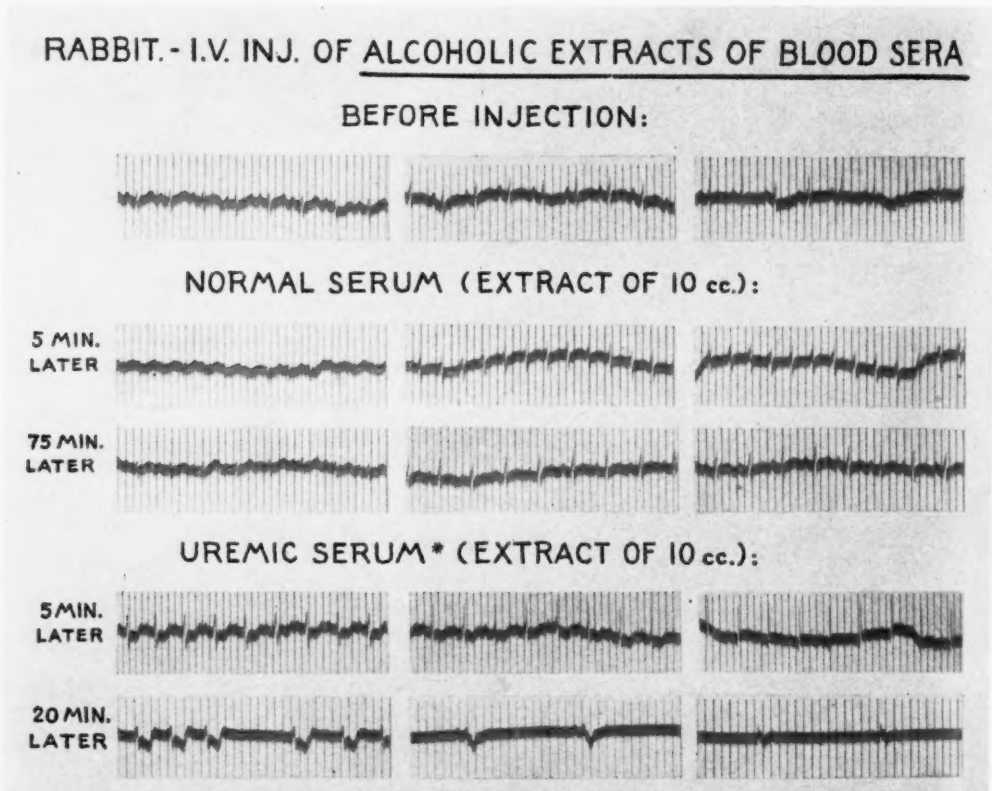
RABBIT. - I.V. INJ. OF ALCOHOLIC EXTRACTS OF BLOOD SERA
BEFORE INJECTION:
NORMAL SERUM (EXTRACT OF 10 cc.):
5 MIN. LATER
75 MIN. LATER
UREMIC SERUM* (EXTRACT OF 10 cc.):
5 MIN. LATER
20 MIN. LATER
* ♂ 34 yrs. - BLOOD AC 305 col.un./cc, NPN 172 mg%, Bl.Pr. 215/130 mm.
PATIENT'S ECG.

Fig. 12.—Effect of normal and of uremic serum upon the rabbit's electrocardiogram. (Intravenous injection of alcoholic serum extracts.) Death followed two minutes after the last electrocardiogram.

One-half to 1 c.c. of alcoholic extract (corresponding to 10 to 20 c.c. of serum) was diluted with a double volume of physiologic saline solution and injected slowly into the ear vein. (Injection of 1 c.c. of undiluted extract from normal serum had resulted in the death of one animal.) Extracts of normal serum (Figs. 11 and 12), although causing moderate tachycardia or bradycardia and some flattening of the T waves, did not produce any other grossly recognizable effects. The electrocardiograms returned toward normal

within an hour or so. Subsequent injection of equal amounts of uremic serum extract (obtained from Cases 9 and 27) was followed within a few minutes by severe anoxic changes of the electrocardiogram (Figs. 11 and 12), flattening and inversion of the T wave, depression of the S-T interval, preterminal bradycardia, and death within eight to twenty-two minutes.

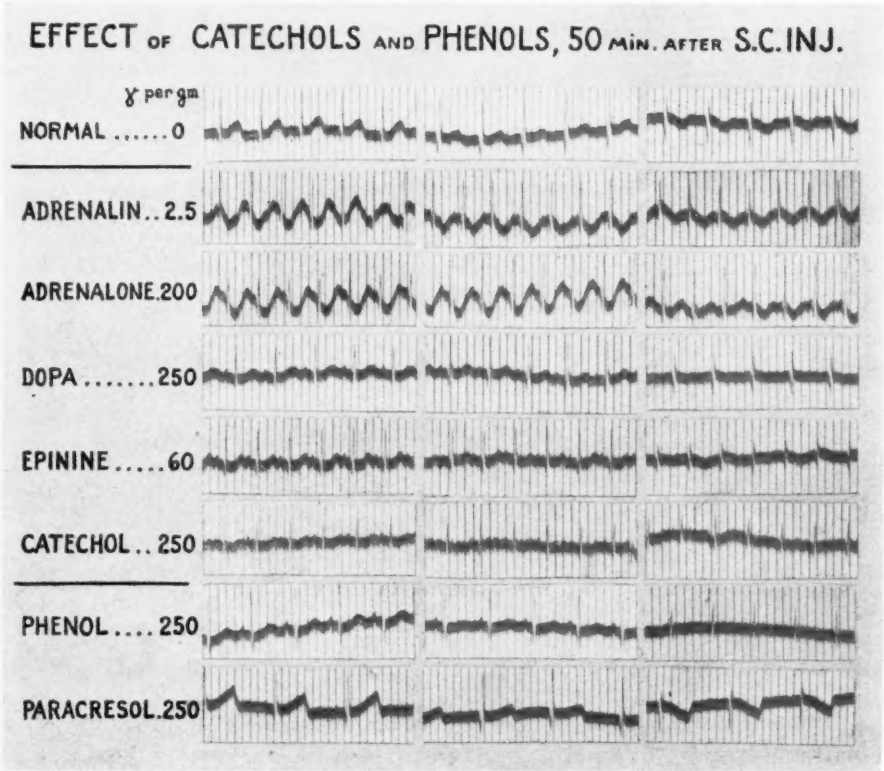

Fig. 13.—Effect of various catechol and phenol compounds upon the electrocardiogram of one rabbit on different days.

For the purpose of comparison with the effects of known catechol compounds, several such substances were injected subcutaneously in large doses into one rabbit (Fig. 13) on different days. In each instance the electrocardiogram was taken fifty minutes after the injection because it was necessary to wait for the disappearance of intense tremors and twitchings which followed some of the injections (particularly of pyrocatechol). Adrenalin and adrenalone produced tachycardia with a depression of the S-T interval and diphasic or inverted T waves. Dihydroxyphenylalanine ("dopa") and epinine (synthetic product) caused some tachycardia and a slight to moderate flattening of the T waves. Pyrocatechol (catechol) also increased the heart rate, reduced the voltage, and caused almost complete flattening or inversion of the T waves (see also Fig. 14 which was made from another animal).

Phenol and paracresol were tested likewise (Fig. 13). Although the latter caused a marked bradycardia and a change in shape of the T waves, no marked flattening or inversion of the T waves occurred.

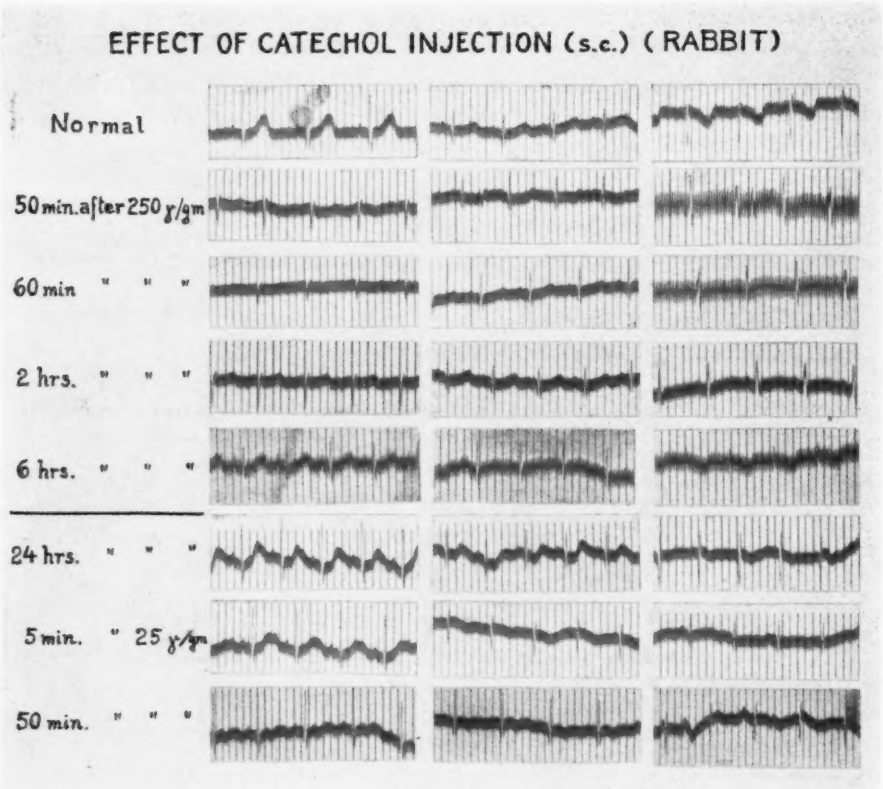

Fig. 14.—Effect of different catechol doses upon the electrocardiogram of a rabbit.

DISCUSSION

Excessive accumulation of catechol compounds (AC) of probably adrenosympathetic origin (adrenalin and related substances) in the blood as well as in the heart muscle was found to be a characteristic feature of uremia (28 clinical cases).

With only few exceptions the uremic patients displayed signs of cardiac failure and marked abnormalities of the electrocardiogram of the anoxic type.

The uremic sera examined showed specific, intensely cardiotoxic properties as manifested by their effects upon the isolated frog heart and upon the rabbit's electrocardiogram. The latter effects were produced by protein-free serum extracts which contained the entire catechol material and which proved rapidly fatal.

Correlation of the clinical, biochemical, and experimental findings suggested the probable identity of the excess catechol material present in the uremic blood and myocardium with at least part of the cardiotoxic substances present in the uremic serum.

In an attempt to substantiate this conception, model experiments were performed with various known catechol compounds, such as adrenalin, adrenalone, dihydroxyphenylalanine, epinine, and pyrocatechol, on the isolated frog heart and on the rabbit's electrocardiogram. By this procedure some of the

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characteristic cardiotoxic effects of the uremic serum could be reproduced: anoxic changes of the electrocardiogram and alterations of cardiac rhythm and contractility, ranging between tachycardia and cardiac standstill and between increase and decrease of cardiac amplitude. All of these partly divergent features are commonly observed in clinical uremia as well.

There is no indication from the chemical findings that adrenalin proper forms a major fraction of the excess catechol compounds accumulated in the uremic organism, but since Richter has shown that adrenalin is normally excreted by the kidneys in a modified form, it appears probable that altered adrenalin and other adrenalin-like substances which are produced by the adrenal medulla and probably by the entire sympathetic nervous system (see Raab,[1, 3] and Cannon and Lissák) are retained in the body in conditions of advanced renal excretory insufficiency.

The general toxic effects of catechol compounds upon the heart muscle are, of course, most strikingly exemplified by adrenalin: stimulation as well as inhibition (Sollmann and Barlow[2]), myocardial anoxia, structural damage and failure (Raab[1]); but in view of the fact that the catechol nucleus is believed to be essentially responsible for the pharmacodynamic effects of adrenalin (Dakin; Tainter; Vaughan, Perkins, and Derbes) and for its high electric potential (Ball and Chen), it appears reasonable to ascribe adrenalin-like cardiotoxic effects also to other biologic catechol compounds as found in excess in the uremic organism.

It must be emphasized, however, that beside catechols, intestinal phenols (bearing only one free hydroxyl group on a benzene ring instead of two) also form a part of the abnormal material which accumulates in the uremic blood. They too, possess certain cardiotoxic properties (bradycardia, decrease of amplitude) and, therefore, must be considered as participating next to the catechols in those chemical mechanisms which bring about cardiac damage and ultimately cardiac death in a large number of uremic patients.

The fatal effect of the accumulation of adrenalin and of other related catechol compounds in the heart muscle above a certain critical level has been discussed elsewhere (Raab[1]) on the basis of experimental and clinicopathologic observations.

SUMMARY

Blood and heart muscle of uremic patients were found to contain excessive amounts of catechol compounds of probably adreno-sympathetic origin.

These findings were roughly paralleled by the presence of anoxic electrocardiographic changes and signs of cardiac failure.

The sera of uremic patients displayed specific, strikingly toxic effects on the isolated frog heart and on the heart of the intact rabbit.

Analogous effects were reproduced experimentally by various known catechol compounds and in part also by phenols.

Both catechol and phenol compounds are believed to participate significantly in the chemical mechanism leading to cardiac failure and death in uremia.

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LABORATORY METHODS

GENERAL

LEUCOCYTOSIS AS AN INDEX OF PYROGENICITY IN FLUIDS FOR INTRAVENOUS USE

E. Gordon Young, Ph.D., F.R.S.C., and F. A. H. Rice, B.Sc., Halifax, N. S.

SEVERAL tests have been proposed within recent years to detect the presence of pyrogenic substances of bacterial origin in distilled water for intravenous use. Welch, Calvery, McClosky and Price[13] have suggested the measurement of rectal temperature in rabbits with a rise of 0.8° C. or more within three hours as indicative of the presence of pyrogen. They have expressed the potency of their standard preparation of *Pseudomonas aeruginosa* in terms of nitrogen and found that intravenous injections of 0.75 to 1.50 mg. invariably elicited a typical response. The limit was given as 0.83 μg. as calculated by us from their data. Lees and Levvy[8] have also made use of rise in rectal temperature in rabbits as a test for pyrogens.

Co Tui, McClosky, Schrift and Yates[5] called attention to the leucopenia in dogs as a criterion of pyrogenicity and accepted a decrease of 5,000 in the white cell count in about forty-five minutes as indicating the presence of pyrogen. Chapman[4] has studied this method in rabbits and found it about as sensitive as that based on temperature but less stringent as to precautions necessary.

The metabolic products of several organisms appear to be capable of inducing the syndrome of nausea, vomiting, defecation, chills and fever. Bourne and Seibert[2] associated the phenomenon in varying degree with several nonchromogenic bacteria from river water, Banks[1] with the chromogenic *Pseudomonas scissa* and *ureae* and Co Tui and Schrift[6] with several pathogenic and facultative saprophytic bacteria. The nitrogen equivalent cannot therefore be taken strictly as an index of pyrogenicity.

The phenomenon is apparently so widespread and of such general importance in intravenous medication that a simple test for the presence of pyrogenic substances is very desirable. This is substantiated by a recent collaborative study in 15 laboratories (McClosky *et al.*[10]). In the course of an investigation of the alleged leucocytic response to allantoin in man the active agent was demonstrated to be pyrogen (Young and Hawkins[14]). The leucocytosis appeared to be detectable in the absence of any subjective symptoms and when no leucopenia or hyperpyrexia was demonstrable. These observations have led us to study the relative sensitivity of these criteria for detecting pyrogenic compounds.

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EXPERIMENTAL

The plan of the investigation was to prepare a sample of pyrogenic water and to inject this into dogs. Their response was to be noted at successive dilutions in terms of objective symptoms, rectal temperature, and white blood cell and differential counts.

A culture of *Pseudomonas aeruginosa*, obtained from our Department of Bacteriology, was grown on nutrient broth for twenty-four hours at 37° C. This was used to inoculate three Kolle flasks containing Difco nutrient agar. After incubation at 37° for forty-eight hours, the growth was washed off with 30 c.c. of redistilled water to each flask. The combined washings were agitated for five minutes in a Waring blender, then incubated at 37° for four days. Sodium chloride (Merck's reagent for biological work) was added to make 0.90 per cent and the solution passed through a medium Berkefeld candle. The filtrate was kept sterile at 4° and used directly for injection as the stock standard. This solution was diluted with fresh triply distilled water which had been distilled from (1) a Barnstead still, (2) dilute acid potassium permanganate, (3) dilute barium hydroxide. The last two distillations were made in all-glass stills with fractionating attachments (Pyrex No. 3360 and Fisher No. 9-107). Sodium chloride was added to make 0.9 per cent, and such diluted solutions were immediately passed through the Berkefeld filter. The stock standard contained 5.60 mg. of nitrogen per 100 c.c. determined by the micro-Kjeldahl method in a Pregl apparatus.

Six dogs, varying in weight from 7 to 23 kilograms, have served as experimental subjects. They have been used in preference to rabbits because of the notorious instability of the latter for experimental purposes. The animals were fed Purina dog chow once a day. Fluctuations in rectal temperature and cell counts were observed frequently during at least one day prior to injection with pyrogen. Blood was taken from the ear for white cell and differential counts. For the latter, Wright's stain was used and 300 to 500 cells were identified each time by cross counting with the aid of a mechanical stage. Injections were made into one of the veins in the hind leg. We made use of sodium amytal (Eli Lilly and Co.) as anesthetic initially but discontinued this practice because in some animals it gave rise to a leucocytosis. Observations were frequently necessary over a period of 16 hours to determine the duration of the leucocytosis and consequently it was not accurately determined in a few cases.

In Table I are shown the results of injecting 20 c.c. of tridistilled water and of an old sample of singly distilled water which had been about the laboratory for several weeks. These may be contrasted with progressive dilutions of the stock pyrogenic standard. A typical response was observed with the latter accompanied by a leucopenia of 4,000 cells below the mean and a leucocytosis of 11,00 cells above it. The amount of nitrogen injected was 280 μg. The leucopenia and hyperpyrexia had disappeared as had any objective symptoms at a dilution of 1:1,000. The leucocytosis was still definite. It was appreciable at 1:5,000 but absent at 1:10,000 based on the reasonable restrictions suggested by Medlar[11] that a significant increase should be more than 50 per cent of the

TABLE I

EFFECTS OF PYROGEN ON WHITE BLOOD CELLS AND TEMPERATURE OF THE DOG

DOG	EXPERIMENT	W.B.C. VARIATION	NEUTROPHIL VARIATION	LEUCOPENIA		LEUCOCYTOSIS		TEMPERATURE VARIATION	HYPER-PYREXIA	REMARKS
				MAX.	DURATION	MAX.	DURATION			
		per c.mm.	%	hr.	hr.	hr.	hr.	° F.	° F.	
A, ♂	Control	8,060- 8,560	60-69	–	–	–	–	101.7-102.0	–	
23 kg.	Redistilled water	8,240- 9,040	68-71	–	–	–	–	101.7-102.0	–	20 ml. injected
	Old distilled water	8,200-12,080	68-74	–	–	5	5	101.3-102.0	–	20 ml. injected
	Pyrogenic water									
	1:0	4,200-19,920	65-92	1	3	7	10	101.6-102.6	0.9	5 ml. injected
	1:10	4,800-19,800	68-90	1	2	9	12	101.6-102.3	0.6	5 ml. injected
	1:1,000	9,010-17,400	65-76	–	–	4	8	101.4-101.5	–	5 ml. injected
	1:5,000	9,000-15,580	67-70	–	–	4	4	101.5-101.7	–	5 ml. injected
	1:10,000	8,200- 9,600	68-70	–	–		–	101.6-101.7	–	5 ml. injected

original total and 10 per cent of the differential picture. The neutrophilia was not as sensitive an index as the leucocytosis. This would mean that a minimum response was obtained with 0.056 μ g. nitrogen, equivalent to 0.35 μ g. pyrogenic protein or 0.015 μ g./kg. body weight. Welch, *et al.*,[13] obtained a minimum response with 0.83 μ g. nitrogen as indicated by rectal temperature. Seibert,[12] however, has recorded an effect with 0.005 μ g. pyrogenic protein. It is to be noted that the level of leucocytosis is approximately proportional to the amount of pyrogen in the more dilute preparations.

Fig. 1 presents the observations in detail of a typical experiment done on Dog A as summarized in Table I. It shows a slight effect on the lymphocytes and a typical response to pyrogen by the leucocytes at a dilution of 1:10 of the standard solution.

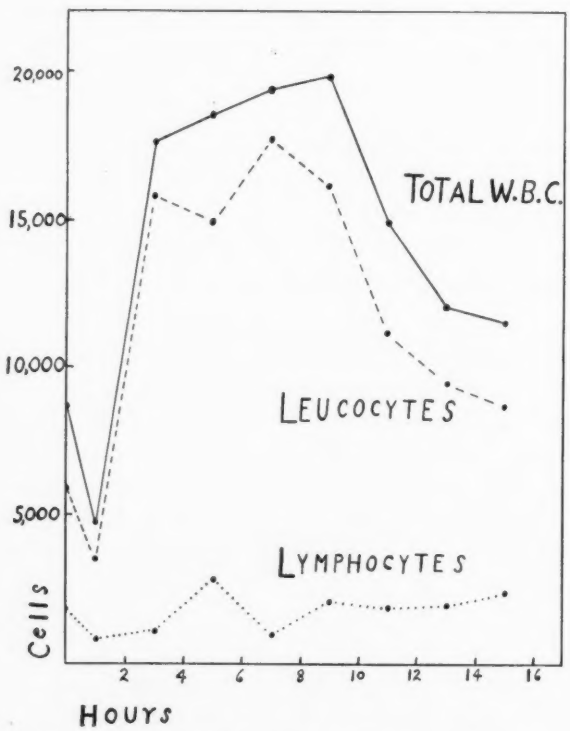

Fig. 1.—Changes in the concentration of the white cell counts after intravenous injection of 5 c.c. of pyrogenic water, diluted 1:10.

Table II presents the evidence from the five dogs used in confirmation of the observations shown in Table I. With the original standard all dogs showed the typical pyrogen syndrome accompanied by a variable hyperpyrexia from 0.5 to 6.1° F., a leucopenia followed by a leucocytosis and neutrophilia. The leucopenia and hyperpyrexia disappeared first with dilution; the neutrophilia was doubtful at 1:1,000; the leucocytosis became doubtful at 1:5,000 but disappeared at 1:10,000. It is therefore to be concluded that in dogs the leucocytosis is the most sensitive index of the presence of pyrogenic compounds. This confirms the observations made previously in man. (Young and Hawkins.[14])

TABLE II

EFFECTS OF PYROGEN ON WHITE BLOOD CELLS AND TEMPERATURE OF THE DOG

DOG	EXPERIMENT	W.B.C. VARIATION	NEUTROPHILE VARIATION	LEUCOPENIA		LEUCOCYTOSIS		TEMPERATURE VARIATION	HYPER-PYREXIA	REMARKS
				MAX.	DURATION	MAX.	DURATION			
		per c.mm.	%	hr.	hr.	hr.	hr.	° F.	° F.	
B, ♂	Control	8,240- 9,280	61-69	–	–	–	–	101.5-101.9	–	
20 kg.	1:10	1,680-17,380	34-92	2	3	8	26*	101.6-102.2	0.5	5 ml. injected
	1:5,000	8,500-12,740	63-70	–	–	3	4	101.7-101.8	–	5 ml. injected
	1:10,000	8,320- 9,280	64-69	–	–	–	–	101.6-101.7	–	5 ml. injected
C, ♀	Control	8,000- 9,300	64-69	–	–	–	–	101.7	–	
7 kg.	1:0	3,460-19,920	32-83	1	3	8	10	101.6-102.2	0.5	2 ml. injected
	1:5,000	8,920-12,020	65-75	–	–	7	4	101.7	–	2 ml. injected
	1:10,000	8,760- 9,900	65-69	–	–	–	–	101.6-101.7	–	2 ml. injected
D, ♀	Control	8,200- 9,000	65-70	–	–	–	–	101.7	–	
7 kg.	1:0	4,200-16,480	63-82	1	3	8	10	101.6-102.2	0.5	1 ml. injected
	1:5,000	8,000-12,220	63-80	–	–	12	3	101.6-101.8	–	1 ml. injected
	1:10,000	8,240- 8,940	65-70	–	–	–	–	101.6-101.7	–	1 ml. injected
E, ♂	Control	8,100- 9,000	63-70	–	–	–	–	101.8	–	
22 kg.	1:0	2,600-24,140	49-93	2	3	10	12	101.9-108.0	6.1	5 ml. injected
	1:1,000	8,670-14,000	65-72	–	–	6	10	101.8-101.9	–	5 ml. injected
	1:5,000	8,200-12,020	65-75	–	–	6	7	101.7	–	5 ml. injected
	1:10,000	8,520- 9,420	66-69	–	–	–	–	101.7	–	5 ml. injected
F, ♂	Control	8,000- 9,200	62-69	–	–	–	–	101.7	–	
20 kg.	1:0	4,080-18,800	40-95	3	4	11	?	101.7-104.2	2.5	5 ml. injected
	1:1,000	8,820-18,400	66-75	–	–	8	12	101.7	–	5 ml. injected
	1:5,000	8,920-13,200	69-70	–	–	7	4	101.8	–	5 ml. injected
	1:10,000	8,320- 9,200	68-70	–	–	–	–	101.7	–	5 ml. injected

It should be pointed out that there are wide differences as to the normal blood picture of the dog in the literature. Downey[7] gives the average white blood cell count at 8,000 and the percentage of neutrophils at 60 to 76. Busch and Van Bergen[3] found the average white cell count to be 9,526 and the neutrophil variation 54 to 74, avg. 65.7 per cent, on 20 dogs. Mayerson[9] placed the white blood cell count at 11,165 and the neutrophils at 74 per cent on 60 dogs. Our short series of six dogs showed a normal fluctuation in the white blood cells of 8,000 to 9,300 and the neutrophils of 60 to 71 per cent.

CONCLUSION

By the intravenous injection of small volumes of water or saline (1 to 20 c.c.) into dogs it is possible to detect the presence of pyrogenic substances at or below the level of concentration which will produce symptoms in man. This is determined most readily by the resulting leucocytosis and progressively by the neutrophilia, leucopenia and hyperpyrexia. White blood cell counts alone, however, will suffice to indicate the presence of pyrogens in objectionable concentrations taking an increase of 50 per cent above the original count as significant on the basis of counts made 3 to 6 hours after injection.

SUMMARY

A study has been made on the dog of criteria indicating the presence of pyrogenic substances in distilled water. Leucocytosis was the most sensitive, measurable in three to six hours after intravenous administration and lasting for many hours. Progressively less sensitive were the neutrophilia, leucopenia and hyperpyrexia.

The least amount of solution producing a positive response contained 0.056 μg. of nitrogen.

The level of leucocytosis was approximately proportional to the amount of pyrogen in the more dilute preparations.

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ON THE MOBILIZATION OF IRON IN HEMOCHROMATOSIS WITH ADMINISTRATION OF VARIOUS CHEMICALS*

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DIFFUSE iron deposits in tissues of the body are pathologic. If in sufficient amount, the presence of iron may be associated with abnormal changes of the organ parenchyma. Atrophy and fibrosis may occur as in the liver and pancreas with hemochromatosis. Chronic inflammatory change may be present in Kaschin-Beck's disease of the joints. The problem of mobilizing abnormal stores of iron and discharging it from the body appears to have had very little attention. This contrasts quite markedly with the efforts put forth toward finding the means of eliminating other metals which are detrimental to the tissues in which they are stored or to the whole body.

In this work some of the agents ordinarily used for the removal of heavy metals from the body were administered, and their effect on the iron balance was measured. The subject was a white man admitted to the hospital with a diagnosis of hemochromatosis in a late stage.

Previously he had been getting along quite well under the care of his family physician. However, while arrangements for metabolic study were being made, his abdomen suddenly became distended and his temperature rose to 105° F. rectally. On instituting stomach drainage he improved rapidly so that by the next day his abdomen was soft and the temperature was 99° F. The liver was quite enlarged and there was some ascites. The diabetes was well controlled with insulin.

After 16 days of medical management he was put on a standard diet of known iron content, with daily collection of urine and stools for chemical analysis. Period separation of feces was made with carmine and charcoal. After a control period of 4 days he was given the following compounds in order, each for a period of 7 days: Sodium thiosulfate, 8½ Gm. daily, divided into three intravenous doses; ammonium chloride, 6 Gm. daily, given orally in three doses; sodium bicarbonate, 9 Gm. daily, in three oral doses; sodium thiosulfate, 17.5 Gm. daily, given intravenously in three doses. The iron intake was kept at a very low level.

Table I shows the details of iron intake and excretion.

Results.—In the control period the excretion of iron in the feces was increased by blood which was found later by chemical tests to be present in the feces of the first two days. Unfortunately it was not feasible to repeat the control period. Blood was not found in the stool in any other period. In Period II there was an appreciable iron retention. The question must be considered, however, of a retention possibly to equalize the iron loss of the preceding period.

*From the Department of Pathology, Northwestern University Medical School.
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TABLE I

PERIOD		TIME	MG. OF IRON INTAKE		MG. OF IRON OUTPUT		MG. OF IRON DAILY EXCRETION		IRON BALANCE DAILY AS MG.
			TOTAL	DAILY	TOTAL	DAILY	URINE	STOOL	
I	Control	4 days	21.24	5.31	48.27	12.07	2.31	9.76	-6.76
II	Sodium Thiosulfate 8½ Gm. daily	7 days	38.0	5.40	21.60	3.08	0.69	2.39	+2.32
III	Ammonium Chloride 6 Gm. daily	7 days	38.0	5.40	31.29	4.47	1.81	2.66	+0.93
IV	Sodium Bicarbonate 9 Gm. daily	7 days	41.4	5.91	40.27	5.75	2.49	3.26	+0.16
V	Sodium Thiosulfate 17½ Gm. daily	7 days	41.4	5.91	64.40	9.23	2.42	6.81	-3.32

In Period III, with the urine made quite acid, there was a very small iron storage. However, the urine was made alkaline in Period IV and again there was slight iron retention. It was decided to push up the thiosulfate dosage to a very high level in Period V. This was done safely, with no evidence of any unfavorable reaction. There was quite an appreciable iron loss with the greater proportion found in the feces. As stated before, blood was not found in the stool in this period.

The urinary excretion of iron in most of the periods was a fairly constant amount. It is sometimes stated to be insignificant as regards total excretion of iron. However, it can be seen in the table that if the figures for urinary iron of the Periods II and V could be exchanged arbitrarily, the significant balances of those periods would be transformed to rather weak ones.

Discussion.—The method of ridding the body of a stored metal may depend not only on the particular metal, but also on the structure in which it is stored. Thus, since lead is deposited in bone along with calcium, it is taken out again by methods which mobilize calcium. Silver deposited in the skin is removed with great difficulty by local treatment because of its insolubility. Bismuth is stored in high amounts in the liver and kidneys and appears to be excreted directly from those organs. Among other metals sometimes found in excess in the body tissues are: mercury, arsenic, zinc, copper, and chromium. There is no known method of accelerating the excretion of any of the above metals by increasing the activity of the liver, kidneys, intestines, or skin.

The direct solution of a metal stored in the body tissues by a chemical given by mouth or intravenously would appear to be difficult. However, this may be accomplished indirectly if the chemical administered reacts with the stored metal to form a soluble salt which is then excreted. This was formerly thought to be the method of action of both potassium iodide and sodium thiosulfate.

It is possible also that salts which supply an excess of available acid or alkaline radicals in the body fluids provide a medium for readier solution of stored metals. This seems to be true of the use of ammonium chloride to mobilize calcium and with it other metals stored in bone.

Iron is often stored as the difficultly soluble hemosiderin. Yet, after a contusion, hemosiderin may be piled up in the tissues in considerable amounts,

but is dissolved and removed within a limited number of days. The iron stores of Addisonian anemia can be taken up and used for building hemoglobin with remission of the disease.

Whether the iron deposited in hemochromatosis can be resorbed either for excretion or hemoglobin formation is not yet known. Even the form in which the iron is stored is not fully understood. Since, however, the sites of greatest iron deposit are subject to profound functional and anatomic change, it would appear important to prevent its deposit or remove it early.

In this work the significance of the negative iron balance of the control period with loss of blood from the intestine could be discussed only if a longer period of loss of a known amount of blood could be correlated with the new formation of hemoglobin from absorbed stores of iron in the tissues. It may be that blood loss furnishes the only feasible method of ensuring a negative balance of iron. Work on this part of the problem is under way.

Summary.—Various chemicals were given to a man with hemochromatosis, over periods of seven days each, with the purpose of attempting to mobilize stored iron. The intake of iron was kept to a known low level, and the output of urinary and fecal iron was determined.

The results do not indicate any striking effect on iron balance by the chemicals given, namely, sodium thiosulfate, ammonium chloride, and sodium bicarbonate.

Sodium thiosulfate in large daily dosage caused a low negative iron balance, but this was outweighed greatly by the iron loss of the control period due to escape of blood in the stool.

Acknowledgment is made here to the valuable support given this work by the Davella Mills Cancer Foundation Fund of the Wesley Memorial Hospital.

A METHOD OF OBTAINING COMPRESSED AIR FOR THE SMALL LABORATORY*

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COMPRESSED air is a necessity in securing a hot flame for pulling or blowing glassware, drying pipettes or other glassware, and cleaning objects of dust particles. Obtaining compressed air for these purposes presents a problem to most workers in small laboratories. Since compression equipment is too expensive for such laboratories, the author has adapted a vacuum cleaner with a few added attachments.

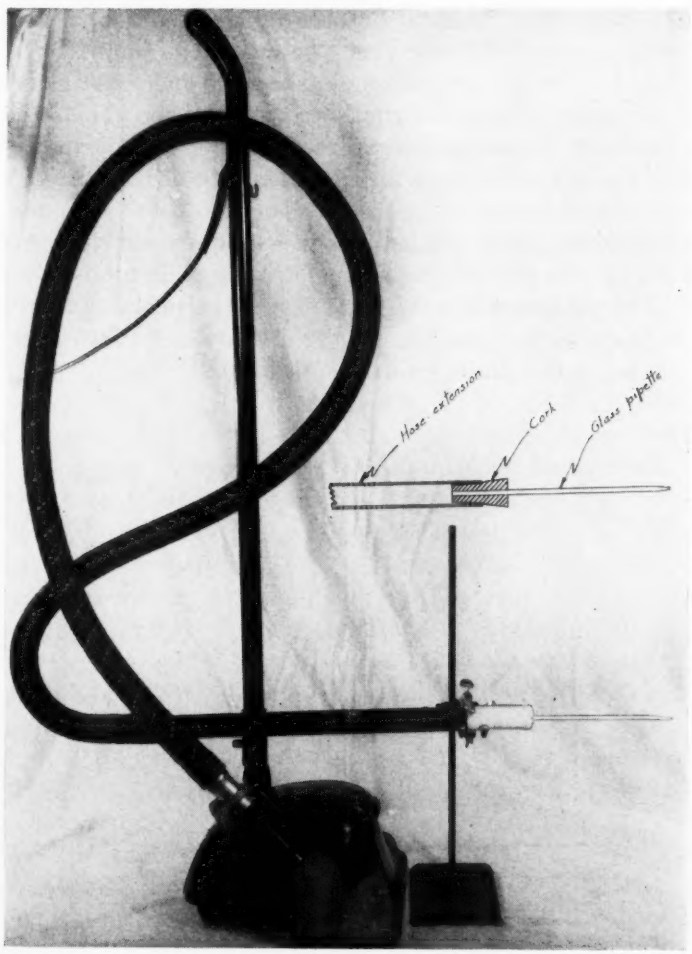

Fig. 1.—Side view of the completed compressor and a detailed drawing of the outlet.

*From the Inland Steel Company Hospital.
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The hose of the blower part of the vacuum cleaner is attached to a *small outlet,* thereby producing a *steady* jet of compressed air. The equipment includes an electric vacuum cleaner with a hose attachment, a cork 1½ inches in diameter, ⅜ inch glass tubing or a 10 c.c. measuring pipette, and a small amount of adhesive tape. The vacuum sweeper must have a blower outlet and hose extension.

The dirt bag of the vacuum cleaner is removed and the hose is attached to the blower outlet. A hole is bored in the cork, into which the glass tubing is fitted. The cork is placed in the exit end of the hose attachment. The exit end of the glass tubing may be small or large. A tube with a small opening is used to produce a small jet of air which may be directed into the flame of a Bunsen burner to produce a blow torch. A 10 c.c. pipette cut and broken at the 2 c.c. mark may be fitted into the cork instead of the glass tube. If the air is to be used for drying glassware or pipettes, a large opening is provided with ⅜ inch glass tubing and a length of rubber tubing. This may be introduced into flasks or attached to pipettes. To obtain an airtight connection, adhesive tape is placed around the end of the hose extension and the adjoining cork. In addition, two pieces of adhesive tape about 1 inch wide and 9 inches in length are placed at right angles to each other on these connections. To do this, a hole is made about midway of one tape which is then slipped onto the tubing. The tape is attached to the cork and to the extension tube of the sweeper, and bound down with the second tape.

An apparatus support stand and a universal extension clamp will hold the hose outlet steady. To prevent the sweeper from picking up dust particles, it is placed on a large cardboard or on a clean floor. The compressor may be turned on and off with the sweeper switch. Fig. 1 shows a side view of the completed compressor and a detailed drawing of the outlet.

A SIMPLE AUTOMATIC PIPETTE

First Lt. Harry A. Kornberg, Sn. C., A. U. S.

THE shortage of man power in many clinical laboratories has made desirable the application of laborsaving devices to routine procedures. In this communication is described such a device which is being used successfully in the author's serology laboratory for the automatic pipetting of colloidal gold.

Although its application has been limited to the rapid delivery of 2.5 c.c. portions, variations in the dimensions of the apparatus will result in the delivery of larger or smaller amounts, extending its use to other procedures in serology as well as to bacteriology and chemistry.

It will be noted that the solution being pipetted contacts no rubber, cork, or other material that may affect the reagent. This is of special importance when colloidal gold is used in the automatic pipette.

Although other designs utilizing the same principle may be used, the one described here was chosen because of its simplicity and the ready availability of material for its assemblage. The colloidal gold solution is contained in a three liter flask fitted with a 2-hole rubber stopper through which passes the delivery tube, bent to the shape illustrated in (*2*) Fig. 1. Since the flask must be two to three feet above the workbench, the descending arm of the delivery tube is approximately twenty inches long. The end of the tube is fitted onto a stopcock (*3*) flanged at one end, such as part of a Van Slyke carbon dioxide apparatus, which, in turn, leads into a Kahn tube (*4*) supported by a clamp attached to a ring stand. Over the side of the Kahn tube is laid the small siphon tube (*5*) prepared as in Fig. 1. Cleaner cuts of aliquots are obtained if the lower end of the siphon tube and the tip of the funnel are heated, dipped into molten paraffin, and the excess is shaken off to give a thin film of wax.

To operate the pipette, the stopcock is opened, air is forced through the short length of tubing fitted into the flask's stopper, and the stopcock is adjusted to a convenient rate of flow. Capillary attraction will allow the siphon tube to operate just before the level of the solution reaches the top of the Kahn tube. Since the rate of flow through the siphon tube must exceed that through the delivery tube, the level of the liquid in the Kahn tube will fall until it reaches the end of the siphon tube when the flow through it will cease. When the liquid again reaches its former height, the siphon will operate as before. Thus, uniform amounts of solution are automatically delivered into the funnel through which they may be directed into the test tubes.

In using the apparatus to pipette many aliquots, we have found it convenient to shove the test tube rack along with one hand, the other moving the funnel suspended by a rubber band down into the mouths of the tubes consecutively as the portions are delivered.

Although it has been found as accurate (0.1 c.c.) as 10 c.c. Mohr measuring pipettes, it is well to check the volumes delivered and at the same time provide

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practice in its use with tap water in the flask. The shorter arm of the siphon tube may have to be lengthened or shortened, depending on whether the quantities expelled are too little or too much.

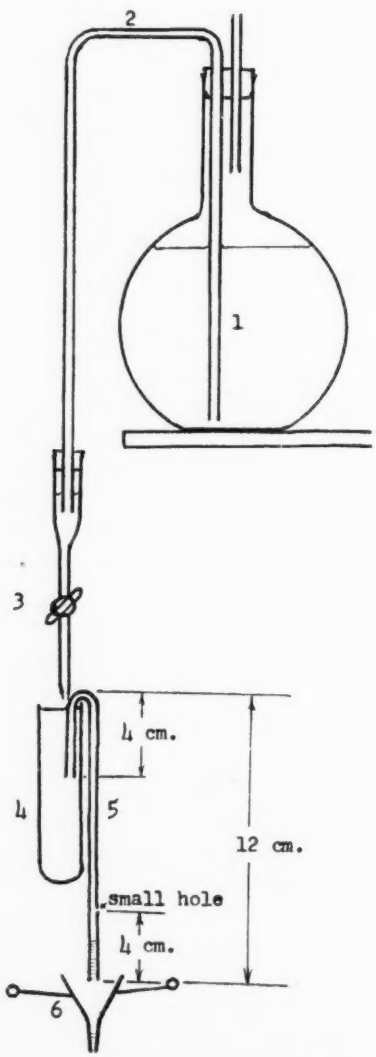

Fig. 1.—*1*, 3 L. flask; *2*, 4 mm. delivery tube; *3*, stopcock; *4*, Kahn tube; *5*, 4 mm. siphon tube; *6*, 2-inch funnel suspended on rubber band from iron ring.

The smooth operation of the pipette depends partially upon surface tension. Hence, the upper part of the siphon tube should be cleaned thoroughly by dipping it into strong cleaning solution and rinsing in tap and distilled waters just before using. A useful refinement is to seal another stopcock in series with the one already present. Thus, one will serve to turn off and on the flow of the solution completely without disturbing the other which is adjusted to the proper rate of flow.

I wish to express appreciation to Miss Sarah G. Miller, Major John T. Cuttino, and Sergeant James Mori for many helpful suggestions.

A SIMPLE APPARATUS FOR PERIODIC TESTING OF P. NOTATUM FLASK CULTURES

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RECENTLY the authors were interested in obtaining small quantities of penicillin broths for a research problem. Production of penicillin was carried out in Erlenmeyer and Fernbach flasks by growing *P. notatum* in surface culture on yeast extract-carbohydrate-mineral salt medium. During these experiments potency tests were made at intervals in order to determine the most appropriate time for harvesting the broth. At first the quantity required for potency test was removed by pipette, but since this necessitated some handling of the flask with consequent disturbance of the culture, the apparatus diagrammed below was devised. This setup is easily constructed from ordinary laboratory equipment, and should be useful generally for investigations on metabolism broths which are to be tested periodically for antibacterial potency and perhaps other properties.

EXPERIMENTAL

The siphons were assembled and attached to 750 ml. Erlenmeyer or 2,800 ml. Fernbach flasks. One hundred or 150 ml. of medium was added to the Erlenmeyer, and 400 or 600 ml. to the Fernbach flasks. Cotton plugs were inserted in the mouths of the flasks and the tops were covered with paper in the usual manner. After the siphon had been started by applying suction to the mouthpiece, the pinchclamp was closed, and the protective sleeve was slipped around the mouthpiece. The entire unit was then autoclaved at 121° C. for twenty minutes. The siphon remained filled after autoclaving, and liquid could be withdrawn from the flask at any time by releasing the pinchclamp.

The sterile medium was seeded by pipette with an aqueous spore suspension of *P. notatum*. Cultures were incubated at 23-24° C. When a quantity of metabolism broth was required for potency testing, a sterile tube, outside diameter 9 mm., previously marked at the 4 ml. level was inserted up to the mouthpiece, and 4 ml. of liquid was withdrawn. This volume which represented about 20% more than the predetermined total volume of the siphon was regarded as the contents of the siphon and was set aside. An additional 2 ml. was then drawn off into a second sterile marked tube for potency testing. In this manner the broth in the siphon from the previous test was removed each time before the quantity for potency testing was taken.

Using routine aseptic precautions, this procedure was repeated eight times over a period of sixteen days on some of the flasks. No obstruction of the

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siphon or contamination of the culture was noted in any instance. The morphological characteristics and rate of growth of the culture appeared the same in flasks with and without the siphon attachment.

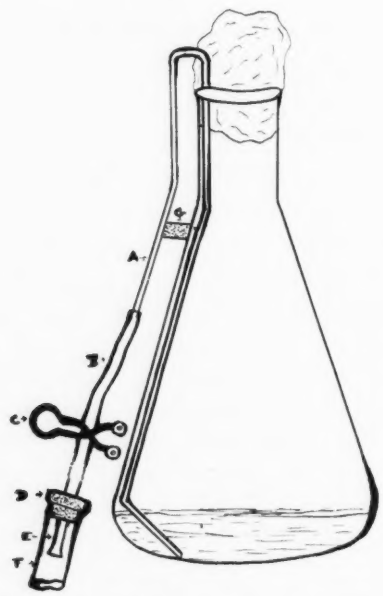

Fig. 1.—Apparatus. *A*, glass tubing, outside diameter 4 mm., wall thickness 1 mm., total length 15 inches (9 inches inside flask, and 6 inches outside); *B*, blood pipette rubber tubing, length 8 inches; *C*, pinchclamp; *D*, rubber tubing, outside diameter 12 mm., wall thickness 3 mm., length 8 mm. Protective sleeve secured on this; *E*, blood pipette mouthpiece; *F*, protective sleeve; glass tubing, inside diameter 11 mm., length 35 mm.; *G*, No. 1 cork stopper as wedge to hold siphon stationary.

SUMMARY

The removal of small quantities of metabolism broth from culture flasks with little disturbance of the culture can be accomplished by the technique and apparatus discussed herein. It seems reasonable to assume that this method might be applied to studies on other fungi which grow similarly where it is desired to test not only the potency of the broth but also changes in pH. By assembling three or four siphons on a single flask, it should be possible to follow pH and potency periodically at various locations and levels in the medium.

MAINTENANCE OF MOISTURE IN BACTERIOLOGIC CULTURE MEDIA

GEORGE H. CHAPMAN, NEW YORK, N. Y.

MANY laboratories prepare culture media and store them for future use but they do not seem to have appreciated the effect of partial dehydration caused by evaporation of moisture from the surface. For example, we have often received cultures on slants of Loeffler's blood serum medium or on blood agar that had become excessively dry. Many microorganisms thrive best in the presence of abundant moisture. Also the concentration of ingredients is affected by dehydration. Hence it is essential to minimize losses of moisture.

In this laboratory, Petri dishes with porcelain covers glazed on the outside are poured approximately 25 ml. each and are stored in an electric refrigerator. The average loss from evaporation, judged by loss of weight, is 0.10 ml. per day. Consequently, the date each medium is poured is noted on a slip of paper which is attached to the set of dishes. When a plate is needed sterile water is added, 0.10 ml. for each day since it was poured, and is spread lightly by a sterile glass spreader. The plate is allowed to stand about a half-hour and is then inverted to drain off any unabsorbed moisture. It is then ready for use.

We have kept different types of blood agar and media containing critical concentrations of bacteriostatic agents for considerable periods of time and have found them entirely satisfactory while other plates from the same lot but not so treated gave poor results.

From the Clinical Research Laboratory, New York.
Received for publication, March 10, 1944.

A VIEWING DEVICE FOR READING KAHN REACTIONS

FLOYD SELL, B.S., DETROIT, MICH.

A VIEWING device is herewith presented which permits the reading of Kahn results without the need of removing the tubes from the Kahn rack. It also permits the reading of individual tubes, when necessary.

The device is so constructed as to permit the transmission of light originating from a daylight fluorescent lamp in such a manner that any precipitate present in the test tubes is illuminated and readily observed in contrast with the clear liquid in which the precipitate is suspended. The device contains shields for the purpose of excluding all extraneous light from the observer's eye. The standard Kahn rack is attached to a tilting lid, thus making possible fine adjustments of the position of the rack, to suit individual workers.

DESCRIPTION OF THE VIEWING DEVICE

The device is enclosed in a hardwood box, measuring approximately 19 × 5 × 6 inches, thus requiring a minimum of space. The box opens with a lid which carries on its underside the holder of the standard Kahn rack. The lid can be fixed by means of a screw at an angle best suitable to the reader. Space is provided at either side of the rack holder where single tubes can be viewed for more detailed study after having been lifted from the rack. A 15 watt daylight fluorescent lamp with its auxillaries is attached to the bottom of the box (Fig. 1). The underside of the lid is painted flat grey and the inside cavity of the box, black, to provide a dark background; a flat black baffle is used to screen out all excess light. The bottom of the box is slightly curved in order to permit some motion of the box and corresponding motion of the fluid in the tubes as it is observed for the presence of precipitates. A diagrammatic plan of the viewing box is presented in Fig. 2.

PROCEDURE OF READING

The rack with tubes containing the completed Kahn test is placed in the rack holder (on the underside of the lid) and the lid is fixed at such an angle that the tubes are almost horizontal to permit observation of a thin layer of solution. The observer directs his eyes at the tubes at such an angle as to receive as little extraneous light as possible (Fig. 3). The tipping of the box causes the fluid in the tubes to undergo slight motion thereby permitting better distinction between precipitates and scratches which may be present on the walls of the tubes. If desired, a single tube may be taken out of the rack and viewed

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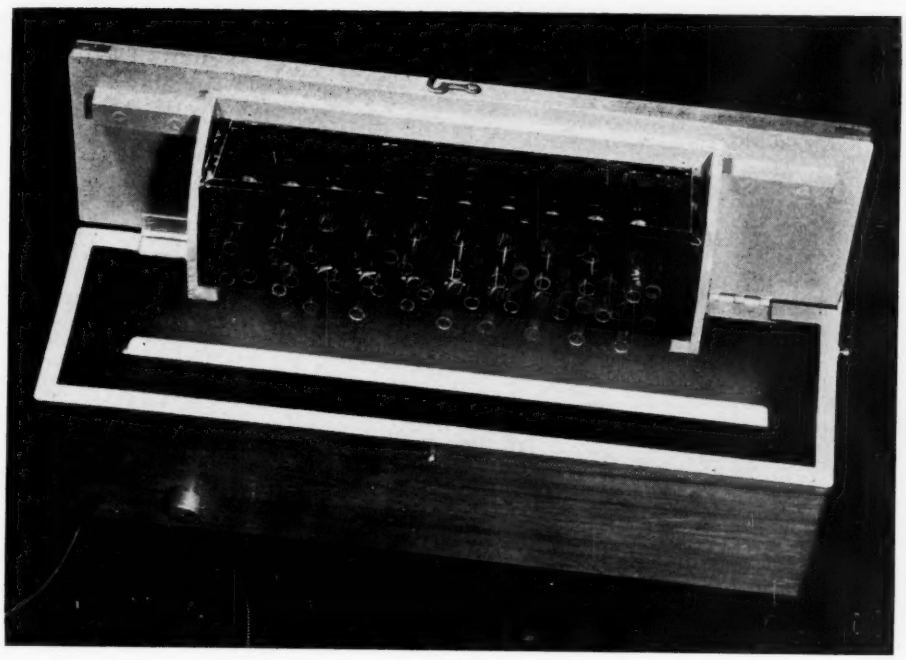

Fig. 1.

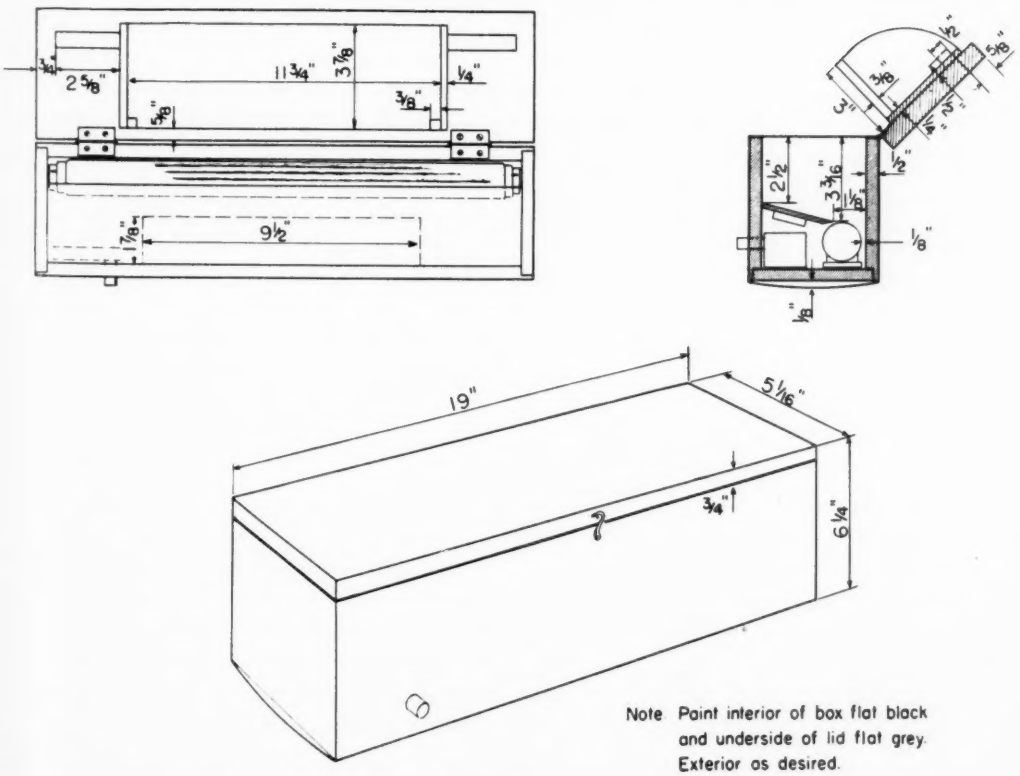
3/4"
2 5/8"
11 3/4"
3 7/8"
1/4"
3/8"
5/8"
1 1/8"
9 1/2"
1/2"
3/16"
3"
2 1/2"
3 3/16"
1 1/8"
1/8"
19"
5 1/16"
3/4"
6 1/4"
Note Paint interior of box flat black and underside of lid flat grey. Exterior as desired.

Fig. 2.

at either sides of the rack in a horizontal position parallel to the axis of the lamp. All readings on this device should be made preferably in a darkened room.

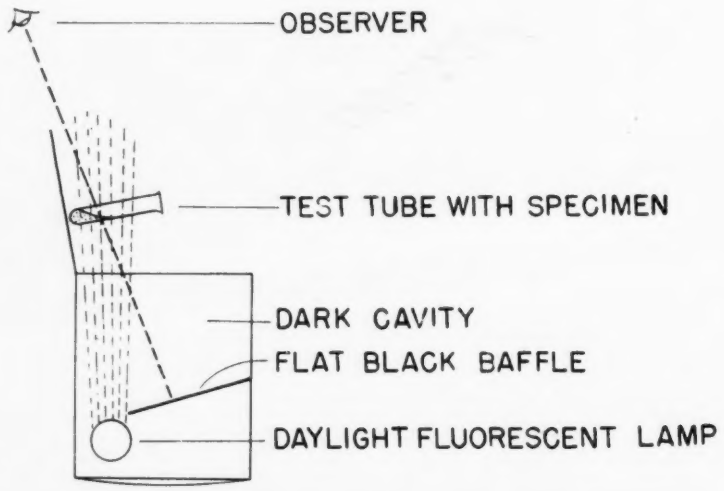

Fig. 3.

SUMMARY

A viewing device for the Kahn test is described which makes possible the reading of precipitation results of an entire Kahn rack or of individual tubes with relative ease.

The author wishes to thank Dr. John Kasper of the Detroit Health Department Laboratories and Dr. R. L. Kahn of the University of Michigan, Ann Arbor, for assistance in this work.

A KAHN VIEWING DEVICE WITH STANDARD ILLUMINATION FOR READING TUBES IN THE RACK*

MAJOR REUBEN CARES, M.C.

THE need for standardizing reading of three-tube flocculation types of serology tests is especially marked when the light conditions can, and do, fluctuate. Many, if not most, laboratories read results by window light. Several textbooks[1, 2, 3] describe how window or other light sources can be adjusted so that some standard or uniform illumination for reading serologic test tubes will result. Obviously, the time of year, or day, the presence or absence of clouds or sunlight will all affect not only the quantity of light but even the nature of light on the tubes. All other conditions being equal with regard to the technique, the marked range in light between a gray overcast winter sky and the brilliant blue summer sky can affect the accuracy of the individual worker in interpreting degrees of precipitation in these test tubes, particularly in borderline positive-negative zones.

The reading devices now available on the market do feature constant illumination for the tubes, but they require removal of the tubes of each test from the standard Kahn rack to the instruments. Where large runs of flocculation tests are performed, most workers choose the easier and more rapid method of reading the entire rack of 10 tests in either window light or in some convenient lamp light. Homemade devices using a uniformly illuminated light-box background are, of course, a substitute for varying daylight source. The usual disadvantage of such an arrangement is reading of the tube suspensions against a bright background. Generally all, or a great part of a rack, is viewed at one time. Eyestrain may cause lower reading efficiency. Since the brightness range between such a light source, and the general laboratory illumination may be too great, the resultant strain may, of course, lower accuracy in reading finer precipitates. Similar strains have been studied by Lowry[4] in connection with viewing motion pictures. A brief discussion of eyestrain factors in laboratory tests requiring fine observation will be found below.

FEATURES OF KAHN RACK VIEWING DEVICE (SEE FIGURES)

The device presented (a) eliminates a variable light source, (b) introduces the feature of side lighting against a dark or black background (admittedly the most critical type of illumination), and (c) allows handling of the tubes without removal from the racks used throughout the procedure. It can easily be constructed out of simple materials and is applicable to any test of the flocculation type requiring the standard Kahn rack. As will be noted in Fig. 1, the light source employs oblique lighting with reference to the optical axis of the laboratory worker. The angle of 13° from the perpendicular is that of maximum

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acuity with reference to any three-tube test in the rack. The first or last of the tests in the rack can be read under the same light conditions as any intermediate test. It will be noted that this particular angle places the back tube, with respect to the light source, exactly between the other two tubes based on projection lines parallel to the axis of the light source. Similarly the front tube with respect to the viewer's line of vision occupies the midpoint between the other two tubes (middle and back tubes) based on projection lines parallel to the optical axis.

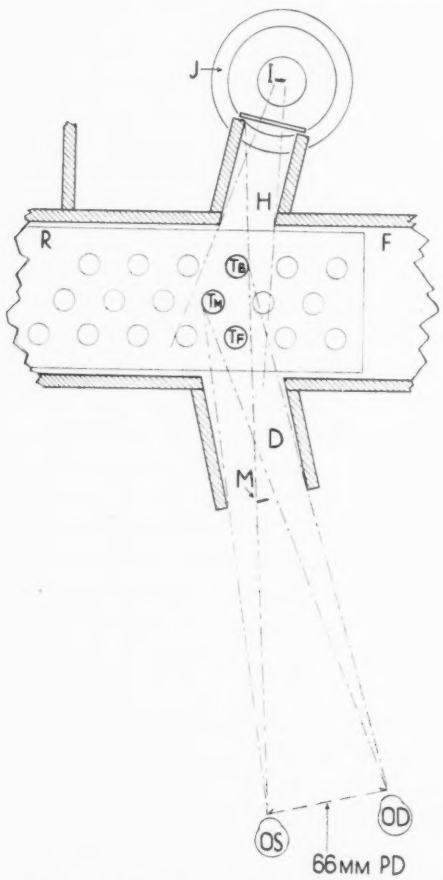


Fig. 1.—Optical features of Kahn viewer.

A—Panel of viewing tube.
B—Front panel of lamp house.
C—Back panel of lamp house.
D—Viewing tube.
E—Adjustable lamp base.
F—Floor of trough for Kahn rack.
G—Lamp housing.
H—Tube for light beam.
I—Standard projection lamp—T8½, 100 w., 115 v.
J—Clear blue glass filter.
K—Set screw for adjustment and centering of lamp filament.
L—Lamp housing vents.
M—Eye shield for view alignment.
R—Standard Kahn rack.
Tf—Front serologic tube (lowest dilution).
Tm—Middle tube.
Tb—Back tube.

Since only one test with its three tubes is read at a time, the field of view of the rack is best confined, as much as is practicable, to the three tubes of this particularly numbered test. Moreover, the tubes examined received the maximum amount of light and so allow visual disregard for the tubes of adjacent tests in the rack.

While the light source, which is fixed, will form a *field of illumination* of constant dimensions, the same does not hold for the *field of view* in the viewing tube. Obviously, with a fixed diameter aperture for the viewing tube, the nearer the eyes the greater is the angle of vision and the more tubes would be visible. The dimensions of the viewing tube, which is adapted for unaided binocular vision, were determined by the average eye-to-object reading distance of 10-12 inches and an average interpupillary distance of 66 mm. Such variations as exist in reading distance or pupillary distance among serological technicians may be readily and almost unconsciously adjusted for by changing eye-to-object distance.

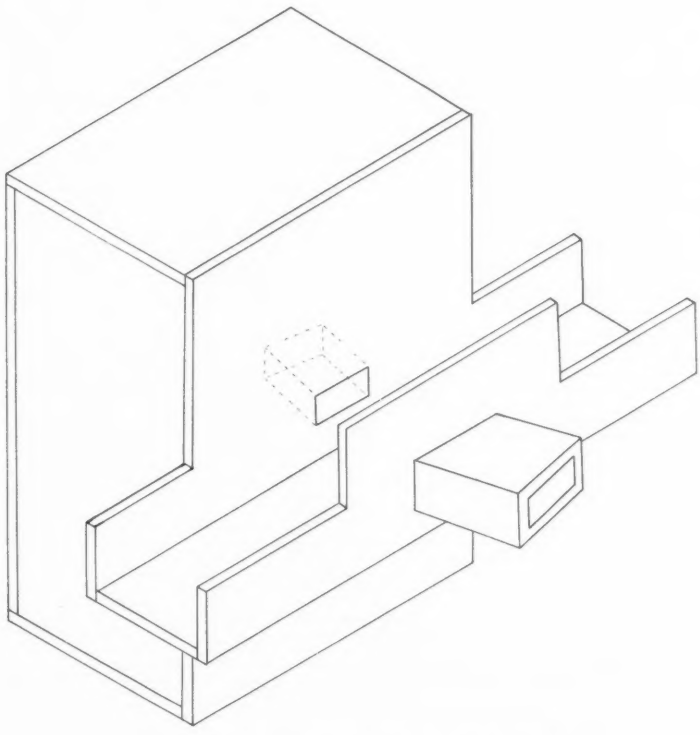
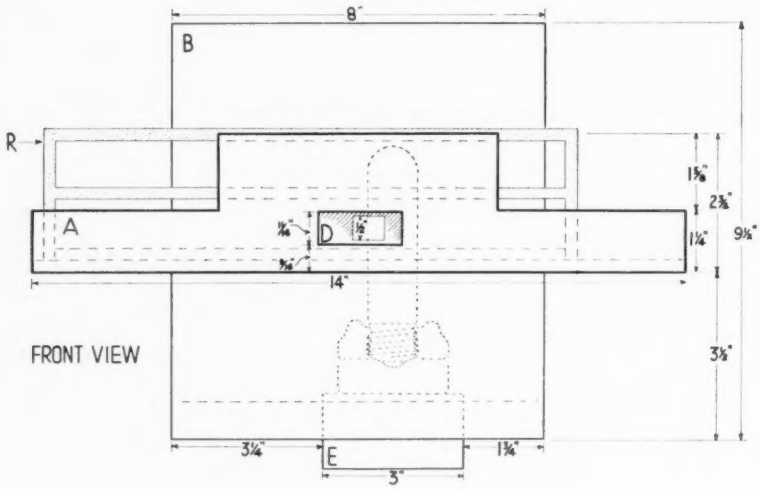


Fig. 2.—Isometric perspective of Kahn viewer.

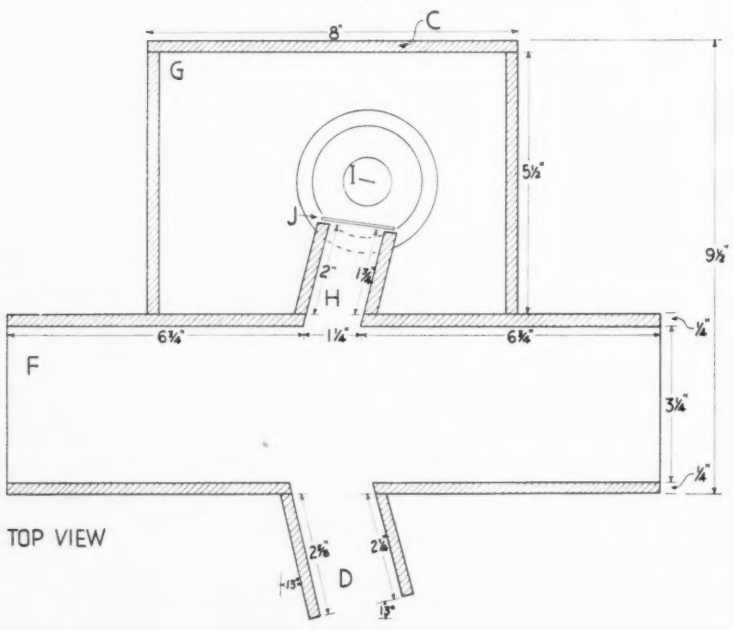
Fig. 2 is a perspective of the viewing device. This consists essentially of a trough through which the Kahn rack may be slid, a viewing tube, and a light source in an appropriate lamp house with illumination directed through a light tube at a fixed oblique angle.

It will be noted in the front view of the device (Fig. 3) that the panel *A* of of the viewing tube reaches only to the top level of the Kahn rack. This is so that all the tubes can be surveyed at a glance for ready reference. The side cut-outs of both the viewing tube panel and the front panel of the lamp house will allow ready grasp of the rack at either end for shaking or other manipulation. In this way almost as much freedom in handling and reading of the ten three-tube tests in a rack is afforded as would be the case in reading by the usual window light.

A few minor refinements are incorporated in this device which require slightly extra equipment or added construction details. The blue glass filter *J* tends to give sharper definition of fine precipitates than straight Mazda light. The lamp block *E* with a set screw *K* allows ready adjustment and centering of



A.

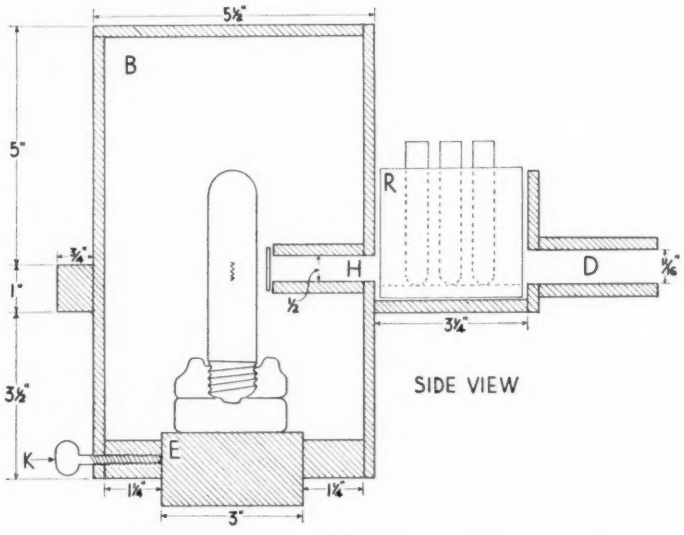


B.

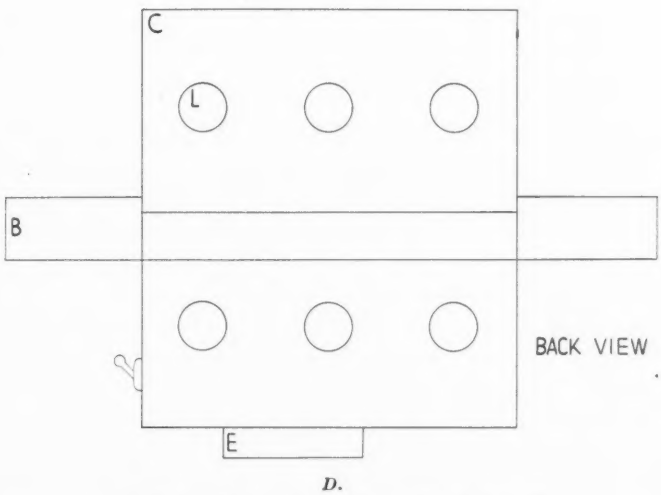
Fig. 3.—Various aspects of device.

the point source of illumination of the concentrated projection filament bulb employed. The lamp housing, being constructed of ¼″ plywood, must be of necessity somewhat more spacious than a metal housing. The latter could be constructed at somewhat greater cost of time and labor.

The table for housing the device (Fig. 4) is designed to be placed on the laboratory bench of 42″ average height. When the viewer is placed into the appropriate cut-out in the table top, the tubes in the rack will be at the average eye level used in reading flocculation tests. The height and outside dimensions of the table may be altered to suit the individual laboratory. The table eliminates the necessity for holding the racks and frees both hands for recording of tests or for related procedures.



C.



D.

Fig. 3 Cont'd.—For legend see opposite page.

SUGGESTED REFINEMENTS AND IMPROVEMENTS FOR VIEWER

Basically the device consists of a directional trough for sliding the Kahn rack into the field of view of a peep tube. A number of refinements for increasing the usefulness of this device are offered. These fall roughly into 3 groups—optical, mechanical, and those related to constructional material.

a. Suggestions for Optical Improvements.

1. A magnifying lens of 2-4 power may be incorporated in the body, end, or base of the viewing tube. A slot for ready removal or insertion of such a lens can be made.

2. The viewing tube, of rectangular or cylindrical form, may be in telescoping sections for ready adjustment by the individual worker. A more complicated binocular tube arrangement can be constructed which should, of course, readily adjust for various pupillary distances.

3. The aperture at the base of the viewing tube, i.e., nearest the Kahn rack, could be widened by a sliding panel to include several sets of tubes for comparison of tests.

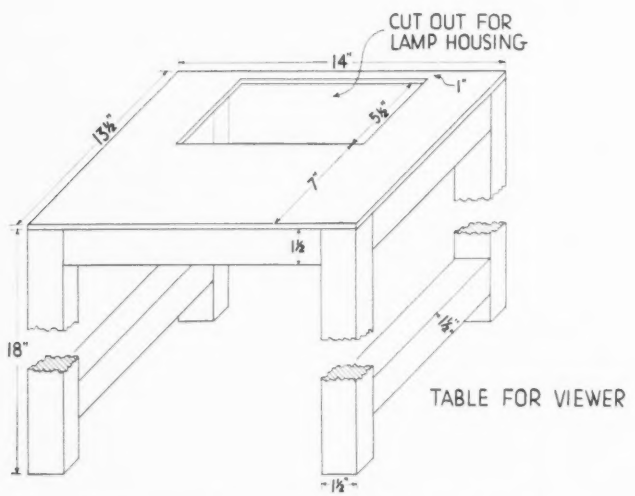


Fig. 4.—Isometric perspective of table for Kahn viewer.

4. The entire device could be further simplified by eliminating the lamp housing *G* and substituting a standard microscope lamp of sufficient light strength. Naturally the angle of the incident light on tubes should be as near as possible to 13° from the perpendicular with reference to the long axis of the trough (and the rack). Otherwise the glare from a more oppositely placed back light may cause visual discomfort to the examiner.

5. A simple frosted glass bulb may be substituted for a projection bulb as a light. However, to prevent the glare from this broader light source the length of the tube for the light beam would have to be increased. This is to insure that the reader does not see the lamp in the field of view of the test tubes. Naturally the longer the light beam tube the less possible will it be to catch a glimpse of the light source through the viewing tube due to the diverging angles (see Fig. 1). The intensity of incident light will fall off if a long light tube is used, so increased wattage may be needed.

6. A precision optical set-up with lamp mirror, designed along film projector lines may be used. It can be incorporated in the lamp housing.

b. Mechanical Refinements.

1. An arrangement for tilting the level floor of the trough for the rack (*F* in Fig. 3*B*) for reshaking tube precipitates could be used.

2. The view tube and tube for the light beam may be made of tubular material with rectangular masks inserted at the tube ends. Since the position of the apertures of both the light and viewing tubes are designed to illuminate only those portions of the three tubes which contain the solutions, reflection or glare from Kahn tube glass above the level of the fluid is minimized. Such rectangular masks of various sizes can be easily constructed so as to slip on readily over the end of the circular or rectangular eye tube.

3. The light shield *M*, as shown in Fig. 1, can be made adjustable to exclude direct rays of the light source from the field of view. It will be noted that the line of vision of the left eye of the observer will permit some direct light glare into this eye. The light shield when in place will not interfere with binocular vision.

c. Materials of Construction.

1. Since the mechanical strength of the viewer requires only sufficient sturdiness to sustain the weight and manipulation of one loaded Kahn rack, alternative materials may be used. All inside dimensions being maintained, some reduction in the bulkiness of the viewer plus its sustaining table can be effected by utilizing sheet metal. Substitutes for plywood may be used.

2. It is even feasible in some laboratories to build-in the apparatus into the wall above the serology workbench so that only a trough and the eye-tube project from the wall above the workbench. An artificial window effect is thus obtained.

3. For those workers who prefer the area surrounding the reading device to be fairly dark, a broad panel, either fixed or removable, can be attached to the back of the lamp housing so no extraneous back lighting would interfere with reading. This would apply, of course, particularly when the apparatus must be placed near or in front of a window necessary for laboratory light or ventilation.

THE PROBLEM OF EYESTRAIN IN READING OF TESTS

It may be appropriate to comment on the variation in visual acuity among laboratory workers from too rapid or too marked a contrast in laboratory illumination. As with other instruments like the microscope, which require concentration on focusing, eyestrain can result from close observation of tiny particles in flocculation tests. Eyestrain has two frequent causes—lack of exercise of lenticular accommodation, and too marked a contrast between general laboratory illumination and illumination within an instrument such as the microscope.[5] Similar conditions obtain when reading fine, colorless precipitates of flocculation tests. If retinal adaptation between low laboratory room lighting and too bright instrument illumination becomes too extreme in range, visual fatigue and subsequent impairment of accuracy in reading flocculation tests may follow. Where one individual may have to read, as is not unusual, up to several hundred sets of Kahn tests, the errors arising from flagging visual accommodation may be an important justification for a second reading of the test by the same individual ten minutes later or by a checking associate.

Since this device is designed to minimize glare and to provide critical standard illumination by side-lighting, eyestrain as a procedural error is largely combated.

SUMMARY

A device is described which can be simply constructed. It has two main advantages:

a. A constant light-source for reading tube flocculation tests.

b. The convenience of using the Kahn rack without removing the individual tubes.

The factor of eyestrain in reading of serologic flocculation tests is discussed. The Kahn viewing device presented attempts to minimize this source of error.

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THE MACROSCOPIC DETERMINATION OF BLOOD GROUPS A AND B

CAPT. LAWRENCE L. SWAN, M.C., A.U.S.

THE present emergency has necessitated the blood grouping of large numbers of individuals, both civilian and military. It is of paramount importance that the methods used be as rapid as compatible with a high degree of accuracy. In Britain blood group determinations have been performed on a large scale also, and a blood transfusion research committee appointed by the Medical Research Council has published recommended methods now to be followed by all transfusion services of Britain.[1]

The method herein described was evolved after many trials and has been found to be highly satisfactory because of its simplicity, rapidity, and accuracy. An effort to determine the accuracy of the typing, resulted in the calculation of the incidence of a large number of blood groups determined by this method.

PREPARATION OF TYPING SERUM

It is well known that the agglutinin titer in individual serum varies markedly, and Thalhimer and Myron[2] found a range from 1:7 to 1:448. Pooling of serum is obviously the most economical of time and equipment to obtain a typing serum of average iso-agglutinin level.

Each type of serum is obtained from 6 or more healthy, young adult males. Blood from each individual is kept separate in 50 c.c. centrifuge tubes and allowed to coagulate in a refrigerator overnight. The tubes are then reamed and centrifuged, and each tube is retyped before the serum is removed and pooled.

A 5 per cent phenol preservative in physiologic saline is added to the serum in the proportion of 1:9. This results in an immediate precipitation of protein which redissolves, to a large extent, upon the complete addition of the phenol. The protein precipitation may be partially eliminated by agitation of the serum as the phenol is added.

The pooled serum is divided into 3 c.c. amounts in Wassermann tubes, tightly stoppered and stored in a refrigerator. One drop of methylene blue is added to each tube of Type A serum for the purpose of differential coloring. Immediately before use, an equal amount of physiologic saline is added to the serum. Specimens of serum stored in excess of 6 months have not decreased in titer or agglutination time.

TITRATION OF TYPING SERUM

It has been found that the results of various techniques of titration vary considerably. The method used here involves pooled sensitive cells as well as pooled serum. The final measure of a successful typing serum is not the titer

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of agglutinins alone, but the rapidity with which macroscopic clumping occurs, for there is little correlation between titer and agglutinating time. At the time blood is drawn approximately a 2.5 per cent suspension of cells in physiologic saline is made from each donor in separate tubes. The content of these tubes is pooled prior to the titration. The cells not incorporated in the clot may also be used after the serum has been removed.

The details of the titration technique are similar to those described by Wiener.[3] The final tube is the dilution in which clumps of 3 to 4 cells may be detected microscopically. The titer of Type A averages 1:32 and of Type B, 1:128 by this method, and definite clumping occurs within 15 seconds.

METHOD OF BLOOD GROUPING

Our experience, and that of others,[4] would indicate that the macroscopic method of blood typing adapts itself satisfactorily to conditions necessitating speed, accuracy and economy of equipment.

A piece of plate glass divided into forty ¾ inch squares is placed on a white background. Two or three drops of diluted serum are placed on the plate in the rows labeled A and B respectively. To this is added a small amount of whole blood from the finger which is pricked by an assistant at the left of the individual adding blood to the serum to make the mixture a light pink, thus aiding in the elimination of pseudo-agglutination. The blood types are read and recorded at the end of three minutes and checked after the elapse of seven minutes.

INCIDENCE OF BLOOD GROUPS

A statistical analysis of the blood groups determined by the described method was found to be within the range accepted by most authorities. A total of 65,350 blood groups were studied. Of this number 61,561 were a heterogenous group of Caucasian males, and 3,789 were Negro males.

The incidence and distribution of blood groups is shown in the accompanying table. The increase in the percentage of Type B at the expense of Type A among those of the Negro race is noteworthy and has been discussed previously by anthropologists.

DISTRIBUTION AND INCIDENCE OF BLOOD GROUPS A AND B

	O		A		B		AB		TOTAL	
		%		%		%		%		%
Negro	1,858	49.03	1,048	27.65	780	20.58	103	2.71	3,789	99.97
White	28,683	46.59	25,042	40.67	5,988	9.72	1,848	3.00	61,561	99.98
Combined	30,541	46.73	26,000	39.92	6,768	10.35	1,951	2.98	65,350	99.98

SUMMARY

1. The preparation of pooled typing serum for the determination of blood groups A and B is described.

2. Phenol in physiologic saline is used as a preservative.

3. The technique of titration involves the use of a pooled suspension of red cells. Type A averages 1:32 and Type B averages 1:128.

4. There is little correlation between agglutinating time and agglutinin titer.

5. The blood groups are determined macroscopically on a plate glass divided into forty ¾ inch squares.

6. A total of 65,350 blood groups determined by this method were studied, and the incidence of Caucasian, Negro and the combined types was within acceptable percentages.

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STABILIZING SERUM FOR BLOOD TYPING

TITUS B. RAY, B.S., AND TRUMAN NELSON, A.B., JACKSON, MICH.

HOSPITAL laboratories, clinics, and private doctors who do a large amount of blood typing, usually have difficulty in preserving blood serum which has been collected and pooled from various sources. The following technique has proved very reliable and satisfactory over a period of the past five years in which it has been employed.

The only special material required for the following procedure is cellophane tubes which may be secured through almost any laboratory supply company for a few cents. There are two types of moisture-proof cellophane tubes which may be used for dehydrating the serum. One, a 29 × 200 mm. tube which is closed at one end; the other, a plain cellophane tubing which may be converted into a U-shaped tube and suspended by clamping both ends to a support. This method of suspension allows free circulation of air during the process of dehydration. Preference is given to dehydration by fanned air for it has been found more satisfactory.

PROCEDURE FOR DEHYDRATING SERUM

1. Collect type two (2) and type three (3) serum over a period of three weeks or more and pool in containers according to the corresponding type.

2. Pour type two (2) serum into one cellophane tube and type three (3) serum into a similar cellophane tube.

3. These tubes are suspended on a support directly in front of an electric fan, approximately two feet away. Leave the tubes in this position until the serum has become completely dehydrated by air from the fan. The dry serum may remain in the cellophane tubes, stored in an ice box until ready for use.

PREPARING SERUM FOR USE

1. Loosen the dehydrated serum from the cellophane tubes by rotating in the palm of hand. Weigh out three (3) grams each of type two (2) and type three (3) dehydrated serum and pour into Pyrex flasks bearing respective labels.

2. Add 35 c.c. of physiologic saline to each flask and rotate five minutes until thoroughly mixed.

3. Place both flasks in H_2O bath at 56° C., rotating occasionally until the serum is completely dissolved. Keep in icebox when not in use. When doing blood typings with dehydrated typing sera, use the same as when employing routine typing sera.

ADVANTAGES

1. Serum prepared this way keeps indefinitely. It has been used up to eight months with accurate results.

2. No preservative is required. Foreign matter does not accumulate as with regular blood typing serum which is pooled from various sources.

3. It is accurate, time saving, very inexpensive, and an advantage to all laboratories, large or small.

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DETERMINATION OF PHENOL COEFFICIENTS IN PRESENCE OF SURFACE TENSION DEPRESSANTS

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USING the Food and Drug Administration (F. D. A.) method[1] for determining phenol coefficients, it was found that Aerosol OT (di-2-ethylhexyl sodium sulfosuccinate) had a distinct action in potentiating the germicidal (or bacteriostatic) action of phenol, cresol, and phenylmercuric nitrate against *Staphylococcus aureus* at 37° C. Details will be published later. Early in the work, it was noticed that although the potentiation was very evident, the results of the inoculation from the medication mixture were very erratic, particularly at the time when killing began to be fairly complete. The results checked poorly in repeat determinations, higher dilutions of the potentiated germicides occasionally appearing to kill the bacteria when lower dilutions did not. Since it was noticed that the drops of mixture taken for inoculation were greatly reduced in size if Aerosol OT was present, the effect of the reduction in volume of inoculum was studied.

The standard loop used for inoculating in the F. D. A. method for phenol coefficients consists of a No. 23 Brown and Sharpe gauge platinum wire, with a loop 4 mm. in inside diameter, bent at a slight angle to the straight part of the wire, which is set in a handle or holder. In making inoculations, the tube of medication mixture (containing 5.0 c.c. of diluted germicide and 0.5 c.c. of a 24-hour culture) is held at an angle so that the loop can be lifted perpendicularly from the surface of the liquid at given intervals of time, before being transferred to the culture tube.

Under normal conditions, it is possible to obtain drops of fairly uniform size and volume. This is not the case in the presence of appreciable amounts of Aerosol OT, since the reduced surface tension causes the formation of drops which are not uniform in size, but which on the average are much thinner and flatter than usual, amounting to possibly 10 to 20 per cent of the volume of normal sized drops. Aerosol OT also usually produces a foam over part of the surface of the medication mixture, and if a drop of inoculum is lifted through this foam, its volume is reduced to a thin film like a soap bubble. When such films break (as is often the case), the remaining liquid amounts to only a few minute droplets along the wire of the loop.

Thus with reduced surface tension, it is apparent that the number of bacteria taken for inoculation is reduced in proportion to the diminished size of the drop. When large numbers of viable bacteria are present, the difference in size of drops is of little importance. The main error occurs after the numbers of bacteria have been greatly reduced by the action of the germicide. If, for example, only 5 viable cells remain in the average normal-sized drop, the inocula-

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tion will give a distinct growth. But if the size of the drop is reduced to 10 per cent of normal volume, then statistically the average drop of reduced size will contain only half a viable bacterial cell, so that some of the drops will contain no living bacteria at all, and will give no growth. Since the drops are not uniformly reduced in size by the Aerosol OT, the irregular results are readily explained.

Although the action of surface tension depressants in diminishing the volume of inoculum is of considerable importance, it seems to have attracted little or no attention. Thus Gershenfeld and collaborators[2-5] who have done most of the studies on potentiation of germicides by surface tension depressants, used the standard F. D. A. methods in most of their work, but do not mention any reduction in drop size.

To determine the size of the standard drop, 10 drops of distilled water were carefully taken on a standard loop, and struck off into a homeopathic vial. The increase in weight of the vial was then determined. The total weight of the first 10 loopfuls was 0.1914 Gm., that of the second 10 loopfuls was 0.2036 Gm. Thus the weight of the average drop is 0.0197 Gm. or very close to 0.02 Gm. (0.02 c.c.).

To obtain 0.02 c.c. of medication mixture for inoculation in the presence of Aerosol OT, sterile 0.2 c.c. Kahn pipettes, graduated to 0.001 c.c., were used instead of the loop in all inoculations. With such pipettes, 0.02 c.c. of medication mixture was easily taken, whether or not Aerosol OT was present.

Using the pipette technique, Aerosol OT did not appear to potentiate the germicides to quite as great a degree as when the loop was used, but different runs gave better checks, so that the results were much more consistent. Thus it was possible to measure the true potentiation, rather than the potentiating action plus the effect of a considerable but variable reduction in the volume of the inoculum.

SUMMARY

In the presence of substances such as Aerosol OT, which materially reduce surface tension, the size of the drop taken for inoculation on the loop in the standard F. D. A. phenol coefficient method is greatly, but not uniformly, reduced. Consistent results are hard to obtain, and apparent potentiation is greater than is actually the case. These difficulties may be overcome by taking 0.02 c.c. of the medication mixture in a sterile pipette for inoculations.

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CHEMICAL

A METHOD FOR THE RECOVERY OF PENICILLIN FROM THE URINE

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IN THE course of introducing penicillin into clinical use in this hospital, it became apparent that the supply would be limited for a considerable time. Since it is known from previous reports (1) that over 50 per cent of a dose of penicillin administered intravenously appears in the urine during the first hour thereafter, we decided to attempt extraction and reclamation of excreted penicillin and to test its efficacy as a therapeutic agent. The principles of extraction are identical with those followed in the manufacture of penicillin. The problem is somewhat easier than that of extraction from cultures of penicillium because the urine contains none of the toxic and pyrogenic fractions which are a source of considerable difficulty. Urinary content of penicillin varies with the size of the dose. When 10,000 units are being administered at intervals of three hours by the intramuscular route, the urine contains from 25 to 40 units per cubic centimeter. With larger dosage, we have found some samples of urine containing 125 units per cubic centimeter. Such concentrations enable one to make a satisfactory extraction of the major portion of penicillin by a single acidification and elution with an organic solvent immiscible with water. For the sake of simplicity, we have confined ourselves to such a procedure, although a small increase in yield results from multiple extractions.

A large container is kept in the icebox for collection of urine from patients under treatment with penicillin. The collections may be made over a period of forty-eight hours without loss of penicillin.

The urine is acidified with concentrated hydrochloric acid and brought to a pH of 2 as determined by the color change produced with methyl violet indicator. Approximately six to seven cubic centimeters of acid are needed for each liter of urine.

Amyl acetate is added to the urine in a proportion of one part to three of urine. A reservoir bottle with an outlet at the bottom serves as a convenient container for mixing. The mixing of the urine and acetate is accomplished by inversion of the bottle twelve or fifteen times. Too much agitation should be avoided to prevent foaming.

The bottle is placed in the icebox for four hours to allow separation of the urine and acetate. The urine is then drawn off through the lower outlet, leaving the penicillin dissolved in the amyl acetate. Any foamy middle layer is passed through several thicknesses of gauze and from it as much amyl acetate recovered as possible.

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The amyl acetate extract is now transferred to a separatory funnel of one liter capacity. Approximately 20 c.c. of phosphate buffer solution are added to it for each liter of amyl acetate. The buffer is prepared by mixing approximately one part of sodium acid phosphate to two parts of *disodium* phosphate by weight, adjusting to pH 7. After mixing the buffer solution with the amyl acetate, some color (representing the pigment which occurs along with penicillin) will be seen to transfer to the buffer solution. The pH of the buffer solution is now adjusted to pH 6.5 by gradual additions of 5 per cent sodium carbonate solution or of two normal sodium hydroxide solutions. By using the latter, less dilution will occur so that a more concentrated final product will result, but more care must be exercised in order not to pass into the alkaline range. The pH is determined by testing of single drops of the solution with brom thymol blue indicator, agitating thoroughly after each addition. When pH 6.5 is reached, practically all the color will have passed from the amyl acetate solution into the buffered aqueous solution. The transfer of penicillin is almost quantitative. The separatory funnel is placed in the icebox for six hours to complete the separation and the amyl acetate is then drawn off. The amyl acetate may be saved and used again.

Additional fractions of amyl acetate extract may be added to the aqueous solution of penicillin in the funnel so that greater concentration may be effected into the same small volume; each time the minimal volume of alkaline solution is added to return the reaction to pH 6.5. With increasing concentration of the extract, the color of the solution approaches that of coffee.

Testing for activity of penicillin is done by the dilution method against staphylococcus H, as described by Hamre, Rake, et al.[2] A satisfactory product is then Seitz filtered into sterile vials. Useful concentrations of three thousand units of penicillin or more per cubic centimeter may be obtained. The yield with this method has been approximately thirty per cent of the amount administered to the patients. For every one million units given the patient, three hundred thousand units can be recovered in a form suitable for re-use.

A test for toxicity is made by injecting one hundred thousand units intravenously into one or more rabbits. Thus far, no toxicity has been encountered. Since the titration of the extract for activity and the testing for toxicity represent the most involved steps in the process, it is suggested that a number of extractions be pooled and concentrated as much as possible and that a sample from the total be titrated and tested. Two hundred cubic centimeters or more of the concentrated deeply colored extract are usually collected by us before testing.

The aqueous solution of the filtered extract is kept in an icebox in vials until used. The extract has been used in doses equivalent in amount to the commercial product in patients with gonorrheal urethritis in this hospital with equal effectiveness and without reaction. Storage for sixty days at –10° C. demonstrated complete maintenance of anti-biotic activity.

Extraction of penicillin from urine and its re-use in patients was reported by Abraham, E. P., Chain, E., et al.,[3] and they noted that pyrogens contained in the original penicillin preparation were removed by passage through the body. Their paper contains no description of the technique of recovery.

SUMMARY

In order to extend the available supply of penicillin, adaptations have been made of the methods employed in penicillin production and a simplified technique is described by which penicillin can be reclaimed from the urine of patients receiving it. Extraction and titration by this method can be performed with apparatus and materials available in an ordinary hospital laboratory. An average yield of 30 per cent of the administered amount has been obtained, and the antibiotic and pharmacologic effects have been identical with those of commercial penicillin.

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THE DETERMINATION OF TOCOPHEROL IN BLOOD SERUM

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MORE determinations of the tocopherol content of human blood serum are needed in studies of vitamin E in human nutrition and in attempts to demonstrate whether or not lack of vitamin E may play a role in certain pathologic conditions. The only figures yet available were contributed by Wechsler, Mayer and Sabotka,[1, 2] who reported comparative studies of the concentrations of tocopherol in the blood sera of normal persons and of patients with amyotrophic lateral sclerosis and with various myopathies. In a recent attempt to make direct application of the procedure described by Mayer and Sabotka,[3] we encountered so many difficulties that it seems worth while to record here the modifications we have found necessary in order to obtain satisfactorily quantitative results.

The method best suited for the determination of small amounts of tocopherol appears to be the photoelectric measurement of the color produced when tocopherol reacts with ferric iron in the presence of α-ά-dipyridyl in an organic solvent. This sensitive color reaction was originally described by Emmerie and Engle[4] and the same colorimetric principle was used by Mayer and Sabotka[3] in their study of blood serum and by Devlin and Mattill[5] and Hines and Mattill[6] for measuring the amount of tocopherol in tissues. The reaction has the advantage of great sensitivity and, at the same time, the disadvantage of lack of specificity for tocopherol. The variety of modifications introduced since the method was originally described represents attempts (a) to work out optimum conditions so that full advantage may be taken of the delicate color reaction, and (b) to isolate quantitatively the tocopherol present in biologic material from other substances which would contribute to or interfere with the amount of color produced in the final colorimetric measurement.

COLORIMETRIC DETERMINATION OF PURE ALPHA TOCOPHEROL

The color reaction, as originally employed by Emmerie and Engle[7] and later used by Mayer and Sabotka,[3] was carried out in a mixture of alcohol and benzene. Although quantitative results were obtained under these conditions when certain precautions were observed, the use of alcohol as a solvent has distinct disadvantages. When ferric chloride and α-ά-dipyridyl are brought together in absolute alcohol, a deep coloration gradually develops, especially on exposure to light. Thus, the two solutions have to be kept separate until used, but even with this precaution there is some development of color when benzene and alcoholic solutions of ferric chloride and dipyridyl are brought together in a blank determination. The substitution of glacial acetic acid for

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absolute alcohol by Devlin and Mattill[5] acting on a suggestion by Merck and Company overcomes this difficulty. A stable reagent can be made by dissolving ferric chloride and α-$\acute{\alpha}$-dipyridyl in glacial acetic acid. The solution has only the yellow color imparted by the ferric chloride and there is no change in color on long standing or exposure to light. Devlin and Mattill[5] carried out the colorimetric step by the addition of this reagent to solutions of tocopherol in Skellysolve E. The reagent is also readily miscible with benzene and no color is produced in blank determinations. Since benzene was more readily available to us it was adopted in preference to Skellysolve E as a solvent for tocopherol in all our analyses. When the reagent is added to a solution of alpha tocopherol in benezene a pinkish orange color develops which reaches maximum intensity in about ten minutes. A Cenco-Sheard Spectrophotelometer was used and readings were made at a wave length of 520 mμ after allowing ten minutes for color development.

Under these conditions the Beer-Lambert law obtained over a range of from 20 to 250 gamma of pure alpha tocopherol in a final reaction volume of 15 c.c. (representing 5 c.c. of a benzene solution of the tocopherol and 10 c.c. of the glacial acetic acid reagent). By plotting the logarithmic values of the transmission against concentration of tocopherol, a straight line of reference was readily obtained or, alternatively, an appropriate function of the extinction coefficient for the ferrous iron-dipyridyl complex could be derived for the calculation of tocopherol in unknown solutions. Occasional rechecking of these values with fresh solutions of alpha tocopherol at intervals of a few weeks has given practically identical figures. This constancy of results has served as reassuring evidence of the reliability of the photometric analysis in the absence of interfering substances.

THE QUANTITATIVE ISOLATION OF TOCOPHEROL FROM BLOOD SERUM

Preliminary to the colorimetric determination the tocopherol must be extracted from blood serum without loss and separated quantitatively from other substances which would interfere with the final color reaction.

Emmerie and Engle, and later, Mayer and Sabotka, used ether for the extraction of tocopherol from blood serum previously treated with formaldehyde, ethyl alcohol, and dilute alkali. Devlin and Mattill used a mixture of Skellysolve and alcohol for tissues treated in a Waring Blendor. In this way they avoided saponification with alkali, which involves the possibility of loss in view of the recognized instability of tocopherol in alkaline solutions. Actually, however, in working with blood serum, we could demonstrate no loss of tocopherol in the brief exposure to dilute alkali involved in the extraction process of Emmerie and Engle and have continued to use this method.

Regardless of which method of extraction is employed, carotenoids and cholesterol are simultaneously extracted and must be removed before the tocopherol can be accurately determined colorimetrically. Selective adsorption of vitamin A and other carotenoids on Floridin X S earth has, in the hands of some investigators, proved a successful means of avoiding the interference from these constituents. Others have found this procedure unsatisfactory, due

possibly to slight differences in the adsorptive properties of different preparations of Floridin earth and to the fact that the fundamental principles governing the success or failure of this selective adsorption have not been accurately defined. Our own experience has been that, despite repeated attempts with several samples of Floridin earth* and careful attention to the details of the procedures described by those who have used it successfully, we have never been able to remove carotenoids without a varying, but always significant, loss of tocopherol. Like Devlin and Mattill, we have found the chemical removal of these contaminants by treatment with 85 per cent sulfuric acid, as described by Parker and McFarlane,[8] a consistently reliable procedure. This treatment does not obviate the interference due to cholesterol, but after the carotenoids have been removed, Devlin and Mattill have shown that cholesterol can be removed without loss of tocopherol by adsorption from benzene on Florisil (Supersorb). After purification by the acid treatment and subsequent passage through adsorbent columns of Florisil, a solution of tocopherol is obtained essentially free from interfering contaminants.

Whatever procedures are adopted, precautions have to be taken throughout the various manipulations to avoid oxidative loss of tocopherol. All evaporations should be carried out *in vacuo*, and flasks, adsorption columns, etc., should be flushed out with nitrogen before the introduction of tocopherol solutions. Direct sunlight should be avoided but the more elaborate precautions of the use of amber glass and a dark room as described by Mayer and Sabotka have not been found to increase the accuracy of the modified procedure which we have adopted.

DETAILS OF METHOD FINALLY USED FOR STUDIES OF BLOOD SERUM

A detailed account of the procedures we have used for the determination of tocopherol in serum follows. Summarized in barest outline, the method combines the extraction process of Emmerie and Engle as modified by Mayer and Sabotka with the Parker and McFarlane method for the removal of vitamin A and other carotenoids, and the adsorptive removal of cholesterol by means of Florisil as described by Devlin and Mattill. The final photometry is carried out on a benzene solution of tocopherol with a solution of ferric chloride and α-ά-dipyridyl in glacial acetic acid.

The following reagents are required:

Peroxide-free ether, redistilled from sodium.

Benzene C.P. reagent.

Petroleum ether, purified as directed by Parker and McFarlane.[8]

95 per cent ethyl alcohol.

Formaldehyde, commercial 37 per cent aqueous solution neutralized with NaOH.

0.2 N KOH in distilled water.

*Kindly furnished for experimental trial by The Floridin Company, Warren, Pennsylvania.

2 per cent KOH in distilled water.

1 per cent (by volume) H_2SO_4 in water containing 1 per cent cadmium sulfate.

0.5 per cent Na_2SO_4 in distilled water.

85 per cent H_2SO_4, 85 c.c. of concentrated H_2SO_4, specific gravity 1.98 diluted to 100 c.c. with distilled water.

Iron-dipyridyl color reagent, 125 mg. of ferric chloride and 250 mg. of α-$\acute{\alpha}$-dipyridyl are dissolved in 500 c.c. of C.P. glacial acetic acid.

Florisil,* purified according to the directions given for Floridin by Emmerie and Engle.[9]

Nitrogen, commercial tank.

PROCEDURE

To a measured 5 to 10 c.c. volume of serum (made to 10 c.c. by the addition of water), in a 250 c.c. glass-stoppered separatory funnel, are added 5 c.c. of 0.2 N KOH, 15 c.c. of formaldehyde solution and 15 c.c. of 95 per cent alcohol, and the mixture thoroughly shaken. Extraction is then carried out with three successive 50 c.c. portions of peroxide-free ether. After each extraction, 10 c.c. of 95 per cent alcohol are added to the aqueous solution preliminary to the next addition of ether. After the third extraction, the aqueous layer is discarded. The combined ether extracts are then washed in a separatory funnel, once with 25 c.c. of 2 per cent KOH, twice with 15 c.c. portions of 1 per cent H_2SO_4 containing cadmium sulfate, and three times with 25 c.c. portions of 0.5 per cent Na_2SO_4. The shaking should be gentle, especially in the final washings, or troublesome emulsions may form. The washed ether is dried for an hour or more over anhydrous sodium sulfate. At this stage the ether extract is perfectly clear and usually has a yellow tint due to the presence of carotenoids.

After drying, the ether solution is quantitatively transferred to a suction flask by filtering through a layer of anhydrous sodium sulfate. Several portions of pure dry ether are used for rinsing the original flask and filter, and these washings are combined with the filtrate. The suction flask is then supported in a warm bath and the ether evaporated *in vacuo*. When only a small volume of ether remains, repeated small additions of benzene are made as the evaporation continues in order to drive off the last traces of water and alcohol. This is conveniently accomplished by using a suction flask fitted with a small separatory funnel for the repeated addition of benzene. (In arranging apparatus for this or any other step in the method, the use of rubber stoppers or connections must be avoided as organic solvents remove material from rubber which later interferes with the accuracy of the method). Usually about 30 to 40 c.c. of benzene are required and this is added in portions of 5 to 10 c.c. until no turbidity results from the addition of benzene to a small residue of fluid in the flask. Evaporation is then continued to dryness. At this point there is a small, yellowish residue left in the flask. The reduced pressure is maintained while the flask

*Purchased from the Floridin Company, Warren, Pa.

is thoroughly cooled, and then an accurately measured 15 c.c. volume of petroleum ether is added which quickly and completely dissolves the residue, and the solution is promptly poured into a glass-stoppered tube which can be centrifuged. Three cubic centimeters of 85 per cent sulfuric acid are added and the tube stoppered and inverted several times until the now brown aqueous layer tends to settle quickly to the bottom of the tube. The stoppered tube is then centrifuged to complete the separation. The clear colorless petroleum ether layer is then transferred as completely as possible to a second glass-stoppered centrifuge tube and washed by inverting several times after the addition of 5 c.c. of 2 per cent KOH. After centrifuging, as large an aliquot as possible of the petroleum ether solution is measured into a small flask. Usually 10 c.c., representing two-thirds of the original 15 c.c. of the petroleum ether solution which contained the entire residue from the sample of serum, can be obtained without difficulty. Obviously all transfers and manipulations of the petroleum ether solution should be carried out as expeditiously as possible and in cool surroundings in order to avoid evaporation of the solvent, and hence change in concentration of dissolved tocopherol, before the aliquot is measured for further treatment.

At this stage the petroleum ether contains tocopherol which is free from vitamin A and carotenoids but is still contaminated with significant amounts of cholesterol. Since the adsorption of cholesterol on Florisil is only satisfactory from a solution in benzene, the aliquot of petroleum ether is evaporated to dryness *in vacuo* and the slight residue is taken up in 5 c.c. of benzene. In the meantime, adsorption columns of Florisil are prepared. Previously purified dry Florisil is placed in glass tubes to form a column approximately 80 by 12 mm. Before use, the Florisil is washed once or twice with pure benzene, and nitrogen gas is run through the column to displace air. The 5 c.c. of benzene solution is then run through the column and is followed by about 25 c.c. of benzene added in portions of 5 c.c. each to the original flask and thence through the column. The combined solution and washings are collected in a small suction flask and evaporated to a volume of about 5 c.c. This is then washed through a second adsorption column of Florisil in exactly the manner just described. Probably for most bloods one passage through Florisil is adequate, but occasionally in dealing with a serum of high cholesterol content a determination was ruined by small amounts of cholesterol which persisted after a single treatment; therefore, the uniform procedure of using two columns was adopted.

When the combined benzene washings from the second adsorption column are evaporated to dryness, the residue is practically invisible. After cooling, this is taken up in 5 c.c. of benzene, and 10 c.c. of the glacial acetic acid reagent are added and the color allowed to develop for ten minutes before the photometric reading is made. A blank solution is prepared simultaneously by the addition of 10 c.c. of the reagent to 5 c.c. of pure benzene. By reference to a calibration curve previously obtained by analysis of pure alpha tocopherol solutions, and appropriate correction for the original volume of serum and the size of aliquot used, the tocopherol content of 100 c.c. of serum can be readily calculated from the observed transmission. As pointed out by Hines and Mattill, the practice of taking a second reading twenty to thirty minutes after the color reagent is

added affords a convenient means of checking the efficacy of the steps for removal of interfering substances. Pure tocopherol solutions show maximum color development in ten minutes, and there is no appreciable change during the next ten to twenty minutes. When impurities are contributing to the color, different time relationships are encountered and there is significant decrease in transmission noted in the later readings as compared to the ten-minute reading. When such differences are noted, the results are unreliable and should not be accepted as true tocopherol values.

Needless to say, before studies of serum are undertaken, blank determinations should be run in which 10 c.c. of water are substituted in place of serum and carried through the entire procedure. Entirely satisfactory blanks should neither cause any development of color with the iron-dipyridyl reagent nor interfere with the development of the full color value when a known amount of tocopherol is measured in the presence of the residue from a blank determination. Actually, a slight amount of color, equivalent to 1 to 3 gamma of tocopherol usually develops in a blank determination in spite of carefully purified reagents. Blanks should be run whenever new lots of reagents are introduced, and a correction made for any slight introduction of extraneous color.

In Table I are collected data which demonstrate the satisfactory recovery of pure alpha tocopherol carried through various steps of the procedure in the manner just described. The figures in the last group also indicate the efficiency of the Parker-McFarlane treatment followed by double adsorption of Florisil in avoiding the interference due to carotenoids and cholesterol.

TABLE I

RECOVERY OF PURE TOCOPHEROL CARRIED THROUGH VARIOUS PROCEDURES

TEST PROCEDURE		TOCOPHEROL RECOVERED (GAMMA)	TOCOPHEROL PRESENT (GAMMA)	PER CENT RECOVERED
α tocopherol in benzene carried through two Florisil columns	(a)	95	100	95
	(b)	102	100	102
α tocopherol carried through acid treatment and two Florisil columns	(a)	100	100	100
	(b)	97	100	97
Mixture of α tocopherol, carotene, and cholesterol, carried through acid treatment and two Florisil columns	(a)	108	100	108
	(b)	97	100	97
	(c)	102	100	102
	(d)	47	50	94

Table II contains the results of preliminary studies with blood serum. Essentially the same values in terms of milligrams of tocopherol per 100 c.c. of a given serum were found whether the determination was run on a 5 or 10 c.c. sample. In spite of the readily reproducible results there should be some reservation in the complete acceptance of the values obtained as the true level of alpha tocopherol. Other tocopherols with less biologic activity than alpha tocopherol give the same color reaction and would not have been removed in the procedures incorporated in the method. Furthermore, although several known interfering substances have been successfully eliminated, there has been no direct proof that others not yet recognized may still be involved. Fairly good evidence that the principal color-producing factor measured is alpha tocopherol is

afforded by the very low values obtained in the serum of rabbits that are seriously deficient in vitamin E, and the prompt increases which are always noted when liberal amounts of vitamin E are restored to such animals. Satisfactory recoveries could be made of alpha tocopherol added to blood serum.

TABLE II

PRELIMINARY BLOOD STUDIES AND RECOVERIES OF ADDED TOCOPHEROL

MATERIAL STUDIED	VOLUME SERUM USED FOR DETERMINATION	TOCOPHEROL FOUND (MG./100 C.C.)	TOCOPHEROL PRESENT (MG./100 C.C.)	RECOVERY OF ADDED TOCOPHEROL (PER CENT)
Human serum A	10	0.97	–	–
Human serum A	5	1.02	–	–
Human Serum A + 1 mg./100 c.c. of added tocopherol	5	2.09	2.00*	109
Human Serum B	10	1.34	–	–
Human Serum B	8	1.32	–	–
Human Serum B + 1 mg./100 c.c. of added tocopherol	9	2.37	2.33*	104
Human Serum B + 0.5 mg./100 c.c. added tocopherol	5	1.78	1.83*	96
Rabbit Serum No. 1. Dying from E deficiency	10	<0.05	–	–
Rabbit Serum No. 2. E deficient diet. Beginning to show weakness	10	0.43	–	–
Rabbit Serum No. 3. Same rabbit as No. 2 after administration of E	8	1.74	–	–
Rabbit Serum No. 4. E deficient for weeks. Marked muscular weakness	8	0.26	–	–
Rabbit Serum No. 5. Same rabbit as No. 4 after heavy E medication	5	2.76	–	–

*Figure based on average figure without additions, plus added tocopherol.

TABLE III

SERUM TOCOPHEROL LEVELS DETERMINED BY THE PROCEDURE DESCRIBED

SUBJECT	AGE (YR.)	SEX	DIAGNOSIS	SERUM TOCOPHEROL (MG./100C.C.)
H. B.	15	M	No disease	0.72
L. J.	11	M	Behavior problem	0.94
J. N.	13	M	Allergy	1.12
M. E.	9	F	No disease	0.88
J. L.	11	M	No disease	0.98
A. G.	5	M	Respiratory infection	1.02
J. C.	5	F	Celiac disease	0.64
F. C.	14	F	Otitis media	0.82
H. B.	9	M	Bronchitis	0.79
D. G.	14	M	Nephritis	1.20
H. J.	21	M	Muscular dystrophy	0.97
H. H.	13	M	Muscular dystrophy	1.02
W. N.	9	M	Muscular dystrophy	1.28
J. A.	6	M	Muscular dystrophy	1.22
J. L.	9	M	Muscular dystrophy	0.73
H. Je.	12	M	Muscular dystrophy	0.80
J. N.	12	M	Muscular dystrophy	0.81

In Table III are presented the results of tocopherol determinations run on the sera of both ill and apparently normal individuals most of whom were in the pediatric age groups. The entire range of values is fairly small and the usual concentration appears to be about 1 mg. of tocopherol per 100 c.c. of serum. In the small group studied there appeared to be no significant difference in the

levels found in sick and well children. Our figures on patients with muscular dystrophy were done in connection with other studies of that disease and their significance has been discussed elsewhere.[10] It is interesting to note the relatively low concentration found in the serum of one child with celiac disease since this patient was found also to have poor absorption of vitamin A. Presumably, conditions unfavorable for the absorption of another fat soluble vitamin would also hinder the absorption of vitamin E.

DISCUSSION

The values for serum tocopherol which we have presented fall within about the same range as those found by Wechsler, Mayer, and Sabotka in adult serum. The fact that two laboratories using different techniques for several steps in the determination have obtained essentially the same results lends considerable support to the probable accuracy of both sets of figures. On this basis there is perhaps little choice between the two methods. In our hands at least, the use of acid for the removal of vitamin A and carotenoids has proved a far less treacherous procedure than selective adsorption on Floridin. Mayer and Sabotka make no mention of the possibility of interference due to cholesterol. Possibly under the conditions of their procedure, cholesterol was removed or had less influence on the final color reaction. Certainly in the procedure which we have used, the removal of cholesterol with the help of Florisil is a vitally important step. At least from the point of view of convenience, the stable glacial acetic acid solution of ferric chloride and α-$\acute{\alpha}$-dipyridyl has great advantages over an alcoholic solution of these reagents.

SUMMARY AND CONCLUSIONS

Modifications of several previously described procedures have been combined in a method which employs the iron-dipyridyl reaction for the colorimetric determination of tocopherol in blood serum.

Data are presented which show (a) alpha tocopherol can be carried through the suggested procedures without significant loss, and (b) that tocopherol can be satisfactorily isolated from substances present in the blood serum which would otherwise interfere with the final colorimetric reaction.

As measured by the method described, the tocopherol content of children's blood serum was found to fall within a small range of values approximating 1 mg. per 100 c.c. of serum.

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EDITORIAL

Editorial Policy

UNDER the guidance of Dr. Victor C. Vaughan, Dr. Warren T. Vaughan, and the Associate Editors who worked so devotedly with them, THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE has served for the past twenty-nine years as a forum for the presentation of clinical investigation and of laboratory techniques. The reasons for beginning the JOURNAL and description of its editorial policy were eloquently stated by the elder Dr. Vaughan in his first editorial: "At present there is a wide chasm between the research man and the practitioner. The work of one supplements that of the other. The world is not benefited nor medicine advanced until the work of both has been done. The basic aim of this JOURNAL will be to bring discovery and its application closer together, to supply the research man with a strictly scientific organ through which he can present the results of his labors, and to suggest to the practitioner how he may use the latest discovery."

Strict adherence to this editorial policy has at times been difficult. With the rapid growth of the medical sciences it often seemed wise to publish manuscripts which were fundamentally biochemical, physiologic, pharmacologic, or bacteriologic in their interest. Their relationship to clinical medicine was not always immediately apparent, although in many instances these investigations later served as the background for new approaches to clinical problems. On the other hand, studies which presented purely clinical observations or case reports often seemed important enough to print even though they described no new principle nor concerned themselves with the pathogenesis or treatment of disease. At first, the section on "Methods" was confined to a discussion of procedures which were of interest to the clinical pathologist or to the practitioner in his own routine laboratory. This policy had to be broadened, however, so that investigators who used either human or animal subjects could be given an opportunity to exchange their experiences with methods of a more purely experimental nature.

There has, therefore, been a gradual broadening of the editorial policy so that papers with a very wide scope of interest were accepted for publication. It is perhaps now necessary to define the boundaries more precisely. The ideal of trying to bring discovery and its application closer together must certainly be preserved. It would seem that this end might best be served: (1) by confining acceptance of experimental papers to those which can properly be included in the broad field of clinical investigation; and (2) by selecting those clinical papers which demonstrate most clearly the application of recent experimental discoveries to the solution of clinical problems. The policy stated above which governs the publication of methods, techniques, and their analyses will be maintained.

C. V. M.

BOOK REVIEWS AND NOTICES

Colorimetric Determination of Traces of Metals. By E. B. Sandell, Ph.D., Assistant Professor of Analytical Chemistry, University of Minnesota, Minneapolis, Minnesota, Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York, 1944. Price $7.00. Cloth with 487 pages.

This book should prove to be a valuable reference work for biochemists and investigators who find it necessary to determine traces of metals in biological materials. It opens with a general discussion of the methods of trace analysis, the problem of separating and isolating traces of substances, and the chemical behavior of the more common colorimetric reagents. The second half of the book is devoted to a presentation of specific methods for individual metals. It is not encyclopedic in its listing of techniques. An attempt is made rather to describe and evaluate a limited number of methods which seem most satisfactory to the author. The treatment of material is so designed as to be of value to industrial as well as to biological chemists. In some instances, consequently, the applications of methods to biological materials are not given in as much detail as a clinical investigator or clinical pathologist might wish. The principles, however, are all clearly stated, and tabulation is made of those substances which interfere with the accuracy of procedures.

The author occasionally makes statements such as the following which make one question his familiarity with the difficulties of doing determinations on biological materials: "The methods now available (for copper) are in the main satisfactory and leave little to be desired." Anyone who has tried to study the copper content of blood realizes how far from the truth this opinion is. However, the reviewer knows of no other work which assembles so compactly and so well the most acceptable methods of colorimetric trace analysis.

C. V. M.

Vascular Responses in the Extremities of Man in Health and Disease. By David I. Abramson, M.D., F.A.C.P. Associate in Charge of Cardiovascular Research, May Institute for Medical Research of the Jewish Hospital, Cincinnati. (Now is a Captain in the Army Medical Corps.) University of Chicago Press, Chicago, Ill. Price $5.00. Cloth with 412 pages.

The book attempts to assemble and correlate information on peripheral vascular disease and vascular responses which has been widely scattered through the literature. The author devotes a large amount of space to the physiological responses of the peripheral vascular tree. Diseases of the venous and arterial systems are evaluated largely from the physiological rather than from the clinical standpoint. Experiments on vascular responses in the limbs of man are extensively considered; animal experiments are described to fill in where data on man are wanting.

The book opens with chapters on the anatomy and physiology of blood vessels. The responses of the peripheral blood vessels to various pharmacological agents, and in systemic diseases are taken up. There follow considerations of "functional" and organic disease. A final section concerns the methods of treatment of vascular disorders.

This volume is well documented, and is readable. References are included at the end of each chapter, and constitute an extensive compilation of the literature. The index is adequate. In the reviewer's opinion, this work should prove of exceptional value to students and clinicians as a brief, authoritative account of vascular problems.

J. R. S.

Stop Worrying and Get Well. By Edward Podolsky, M.D. Bernard Ackerman, Inc., 381 Fourth Avenue, New York 16, New York. Price $2.00. Cloth with 124 pages.

CORRESPONDENCE

To the Editor.—In our recent paper entitled "The Apparent Advantage of Frequently Administered Quinine in Avian Malaria Infections" in the JOURNAL, January, 1944, page 43, we made the statement that we were unaware that frequent, to say nothing of continuous, therapy with quinine had ever been given a trial in acute malaria infections in the human being, and that we, therefore, sought to investigate the possible advantages of such a method of treatment by putting the matter to experimental trial in avian infections in the laboratory. Our attempt to effect continuous administration of solutions to canaries having met with failure, we were obliged to resort to administration of the quinine by mouth at two-hour intervals around the clock for the four days during which the therapeutic trials lasted. We have now received a very kind letter from Col. Charles F. Craig, the eminent malariologist and editor of the *American Journal of Tropical Medicine*, who states his feeling that in our search of the clinical literature, we overlooked the fact that frequent quinine dosage was employed in man many years ago and that the matter is specifically discussed in the publications of Marchiafava and Bignami and of himself. In our copy of Marchiafava and Bignami, that was reprinted from *Twentieth Century Practice* for the use of the U. S. Army in 1900, we have failed to find very specific evidence that the Italian authors did use quinine at frequent intervals in the attempt to accomplish a somewhat "continuous" type of therapy, but there can be no doubt that Col. Craig did report very favorably upon a frequent-administration method of treatment in his work *The Malarial Fevers, etc.*, published in 1909. Among his several references to this type of treatment, there appears (page 373) the following unequivocal statement ". . . it is necessary to give quinine in divided doses of from 0.3 to 0.6 Gm. (5 to 10 grains) at intervals of four to six hours until the temperature reaches normal, and to increase the number of doses if experience shows that

the quantity advised is insufficient." It was our misfortune not to have seen this statement in Col. Craig's valuable book before our paper appeared, and we are therefore writing this letter to place on record our recognition of the fact that Col. Craig did indeed advocate the frequent administration of quinine a great many years ago. If there should be any interest in the matter of priority in recommendation of such a therapeutic approach in malaria, certainly Col. Craig and not ourselves deserves the credit of having first drawn attention to the possible merit of this type of procedure. In passing, however, it might just be of interest to note that possibly the superiority of the method had been attested and forgotten long before Col. Craig introduced it, for Boyd in the A.A.A.S. Symposium on Human Malaria, page 5, 1941, writes as follows: "The earliest report of its employment in the United States that has come to our attention is by Henry Perrine (1826), a physician of Natchez, Mississippi. He employed from six to twelve grains every two to three hours at any period of the fever continued until its symptoms in pulse and skin were subdued, repeating if the return of the fever was suggested."

As a matter of fact, it was not a frequent-dosage method in which we were primarily interested in our work but an attempt to study a continuous method of administration, and it is our hope that a continuous method as advocated in our paper will be given clinical trial with proper controls by those in a position to do so.

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